A

Patent
Attorney's Docket No. 012712-813

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

REQUEST FOR FILING CONTINUATION/DIVISIONAL APPLICATION UNDER 37 C.F.R. § 1.53(b)

Box PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

This is a request for filing a [X] continuation [ ] divisional application under 37 C.F.R. § 1.53(b) of pending Application No. 08/742,480 filed on November 1, 1996, for CD40CR RECEPTOR AND LIGANDS THEREFOR, by the following named inventor(s):

(a) Full Name Randolph J. NOELLE

(b) Full Name _____

(c) Full Name _____

[X] The entire disclosure of the prior application from which a copy of the oath or declaration is supplied herewith is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

[X] This application is being filed by less than all the inventors named in the prior application. In accordance with 37 C.F.R. 1.63(d)(2), the Commissioner is requested to delete the name(s) of the following person or persons who are not inventors of the invention being claimed in this application.

(a) Full Name Alejandro ARUFFO

(b) Full Name Jeffrey LEDBETTER

(c) Full Name Ivan STAMENKOVIC

[ ] This application is being filed by more than all the inventors named in the prior application. In accordance with 37 C.F.R. 1.63(d)(2), the Commissioner is requested to add the name(s) of the following person or persons who are inventors of the invention being claimed in this application.

(a) Full Name _____

(b) Full Name _____

(c) Full Name _____

1. [X] Enclosed is a copy of the prior Application No. 07/835,799 as originally filed on February 14, 1992, now abandoned, including copies of the specification, claims, drawings and the executed oath or declaration as filed.

2. [ ] Enclosed is a revised prior application and a copy of the prior executed oath or declaration as filed. No new matter has been added to the revised application.

3. [ ] _____ statement(s) claiming small entity status [ ] are enclosed [ ] were filed in prior Application No. __, filed on __.

4. [X] The filing fee is calculated below [X] and in accordance with the enclosed preliminary amendment:

CLAIMS					
	NO. OF CLAIMS		EXTRA CLAIMS	RATE	FEE
Basic Application Fee					$760.00 (101)
Total Claims	72	MINUS 20 =	52	x $18.00 (103) =	$936.00
Independent Claims	12	MINUS 3 =	9	x $78.00 (102) =	$702.00
If multiple dependent claims are presented, add $260.00 (104)					$260.00
Total Application Fee					$2,658.00
If small entity status is claimed, subtract 50% of Total Application Fee					
Add Assignment Recording Fee of if Assignment document is enclosed					
TOTAL APPLICATION FEE DUE					**$2,658.00**

5. [ ] Charge $ _____ to Deposit Account No. 02-4800 for the fee due.

6. [X] A check in the amount of $ 2,658.00 is enclosed for the fee due.

7. [X] The Commissioner is hereby authorized to charge any appropriate fees under 37 C.F.R. §§ 1.16, 1.17 and 1.21 that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800. This paper is submitted in triplicate.

8. [X] Cancel in this application original claims 1-41 of the prior application before calculating the filing fee.

9. [X] Amend the specification by inserting before the first line the sentence: --This application is a [X] continuation, [ ] divisional, of Application No. 08/742,480, filed November 1, 1996, in turn a continuation of Application No. 08/338,975, filed November 14, 1994, in turn a continuation of Application No. 07/835,799, filed February 14, 1992, now abandoned.--

10. [ ] Transfer the drawings from the pending prior application to this application and abandon said prior application as of the filing date accorded this application. A duplicate of this paper is enclosed for filing in the prior application file. (May only be used if signed by person authorized under 37 C.F.R. § 1.138 and before payment of issue fee.)

11. [X] New drawings are enclosed.

12. [ ] Priority of Application No. __ filed on _ in __ (country) is claimed under 35 U.S.C. § 119.

[ ] The certified copy of the priority application
[ ] is enclosed
[ ] was filed on __ in prior Application No. __, filed on __
[ ] has not yet been filed.

13. [X] A preliminary amendment is enclosed.

14. [ ] A General Authorization for Payment of Fees and Petitions for Extensions of Time.

15. [X] Also enclosed is a copy of the Order in Interference No. 104,415 and a copy of U.S. Patent No. 5,993,816.

16. [X] The power of attorney in the prior application is to E. Joseph Gess, Burns, Doane, Swecker & Mathis, L.L.P..

a. [X] The power appears in the original papers in the prior application.

b. [ ] Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.

c. [ ] Recognize as Associate Attorney __.

d. [X] Address all future communications to: (May only be completed by applicant, or attorney or agent of record.)

E. Joseph Gess
BURNS, DOANE, SWECKER & MATHIS, L.L.P.
P.O. Box 1404
Alexandria, Virginia 22313-1404

December 20, 1999
Date

By: Robin L Teskin
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ADDRESS OF SIGNATOR:

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[ ] inventor(s)
[ ] assignee of complete interest
[ ] attorney or agent of record
[X] filed under 37 C.F.R. § 1.34(a)

Patent
Attorney's Docket No. 012712-813

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	
	)	
Randolph J. NOELLE	)	Group Art Unit: 1806
	)	
Application No.: Unassigned	)	Examiner: Unassigned
	)	
Filed: December 20, 1999	)	
	)	
For: CD40CR RECEPTOR AND LIGANDS THEREFOR	)	

REQUEST FOR INTERFERENCE BY APPLICANT PURSUANT TO 37 C.F.R. §§1.607 AND 1.608, AND PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified application, kindly amend the application as follows:

IN THE TITLE

Please delete the current title and substitute the following therefor:

--USE OF ANTIBODIES THAT SPECIFICALLY BIND

CD40CR (CD40 LIGAND) TO INHIBIT HUMORAL IMMUNITY--.

IN THE SPECIFICATION

At page 31, after the last line of text, insert the following:

--The hybridoma identified in this application as MR1 was deposited on May 22, 1992 with the American Type Culture Collection, ATCC, International Depository Authority, 12301 Parklawn Drive, Rockville, Maryland 20852, in compliance with the Budapest Treaty, and accorded Accession Number ATCC HB 11048. All restrictions as to the availability to the public of the hybridoma cell line MR1 will be irrevocably withdrawn upon issuance of a United States Patent to this application. Also, access to the MR1 cell line will be available to the Commissioner during the pendency of this patent application or to one determined by the Commissioner to be entitled to such cell line under 37 C.F.R. §1.14 and 35 U.S.C. §122.--

IN THE CLAIMS

Kindly cancel all of Claims 1 through 41, and substitute the following claims therefor.

--42. A method for inhibiting a humoral immune response comprising contacting T-cells with an antibody that binds specifically to a protein specifically recognized by monoclonal antibody MR1 produced by the hybridoma having ATCC Accession No. HB 11048.

43. A method for inhibiting a humoral immune response comprising contacting T-cells with an antibody that binds specifically to CD40CR.

44. A method for inhibiting immunoglobulin production comprising contacting T-cells with an antibody that specifically binds to a protein specifically recognized by monoclonal antibody MR1 produced by the hybridoma having ATCC Accession No. HB 11048.

45. A method for inhibiting immunoglobulin production comprising contacting T-cells with an antibody that specifically binds to CD40CR.

46. A method for inhibiting activation of B-cells comprising contacting T-cells with an antibody that specifically binds to a protein specifically recognized by monoclonal antibody MR1 produced by the hybridoma having ATCC Accession No. HB 11048.

47. A method for inhibiting activation of B-cells comprising contacting T-cells with an antibody that specifically binds to CD40CR.

48. A method for inhibiting a humoral immune response in an animal comprising the step of administering to the animal in an amount effective to inhibit the humoral immune response, an antibody or fragment thereof that binds specifically to a protein specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession No. HB 11048.

49. A method for inhibiting a humoral immune response in an animal comprising the step of administering to the animal, in an amount effective to inhibit the humoral immune response, an antibody or fragment thereof that specifically recognizes CD40CR.

50. A method for inhibiting immunoglobulin production in an animal comprising the step of administering to the animal, in an amount effective to inhibit immunoglobulin production, an antibody or fragment there of that specifically binds to a protein specifically recognized by the hybridoma having ATCC Accession No. HB 11048.

51. A method for inhibiting immunoglobulin production in an animal comprising the step of administering to the animal, in an amount effective to inhibit

immunoglobulin production, an antibody or fragment thereof that specifically recognizes CD40CR.

52. A method for inhibiting activation of B-cells in an animal comprising administering to the animal, in an amount effective to inhibit activation of B-cells, an antibody or fragment thereof that specifically binds to a protein specifically recognized by monoclonal antibody MR1 produced by the hybridoma having ATCC Accession No. HB 11048.

53. A method for inhibiting activation of B-cells in an animal comprising administering to the animal, in an amount effective to inhibit activation of B-cells, an antibody or fragment thereof that specifically recognizes CD40CR.

54. The method of any one of Claims 42 through 53, wherein the antibody or fragment thereof is selected from the group consisting of monoclonal antibodies, chimeric antibodies, human antibodies, and fragments thereof.

55. The method of any of Claims 42 through 53, wherein the antibody or fragment thereof is conjugated to another moiety selected from the group consisting of an enzyme, toxin, growth factor, lymphokine, anti-proliferative agent, alkylating agent,

anti-metabolite, antibiotic, vinca alkaloid, platinum coordinated complex, radioisotope, and fluorescent compound.

56. The method of any one of Claims 42 through 53, wherein the antibody is conjugated to a therapeutic agent.

57. The method of any of Claims 48 through 53, wherein the animal is a mammal.

58. The method of any of Claims 48 through 53, wherein the animal is a human.

REMARKS

By the present amendments, new claims have been presented in favor of the original claims (which are cancelled), all of which correspond to the same invention claimed in Lederman et al, 5,993,816, issued on November 30, 1999. Specifically,

Claims 42 and 43 correspond to Claim 1 of Lederman '816;

Claims 44 and 45 correspond to Claim 2 of Lederman '816;

Claims 46 and 47 correspond to Claim 3 of Lederman '816;

Claims 48 and 49 correspond to Claim 4 of Lederman '816;

Claims 50 and 51 correspond to Claim 4 of Lederman '816;

Claims 52 and 53 correspond to Claim 5 of Lederman '816;

Claim 54 corresponds to Claim 7 of Lederman '816;

Claims 55 and 56 correspond to Claims 9 and 10 of Lederman '816;

Claim 57 corresponds to Claim 13 of Lederman '816; and

Claim 58 corresponds to Claim 14 of Lederman '816.

Specific support for the newly-submitted claims may be found in the as-filed application as follows:

Claims 42, 43: Section 5.4 at pages 17-19, original Claims 22, 24, 27, 28, 29, 30, 31, 32, 37; Section 6.24 at pages 28-31, et seq.

Claims 44, 45: Section 5.4 at pages 17-19, original Claims 22, 24, 27, 28, 29, 30, 31, 32, 37; Section 6.24 at pages 28-31;

Claims 46, 47: Section 5.4 at pages 17-19, original Claims 22, 24, 27, 28, 29, 32, 37; Section 6.24 at pages 28-31;

Claims 48, 49: Section 5.4 at pages 17-19, original Claims 22, 24, 27-32, 37; Section 6.24 at pages 28-31;

Claims 50, 51: Section 5.4 at pages 17-19, original Claims 22, 24, 27-32, 37; Section 6.24 at pages 28-31;

Claims 52, 53: Section 5.4 at pages 17-19, original Claims 22, 24, 27-32, 37; Section 6.24 at pages 28-31;

Claim 54: the specification at page 12, lines 1 to page 13, line 21, et seq.;

Claim 55: the specification at page 11, lines 29-35, et seq.

Claim 56: the specification at page 11, lines 29 to 35, et seq.

Claims 57, 58: original Claims 30-32, 37, 38, 39, and Section 6.24 of the specification (*et seq.)*.

Thus, it can be seen that all of the newly-submitted claims are directed to methods of using antibodies specific to CD40L (also known in the art as CD40CR, gp39, CD154, 5c8 antigen, TBAM) to inhibit humoral immunity, immunoglobulin production and B-cell activation. Such inhibition results because this antibody binds CD40L, which is an antigen expressed on activated T-cells that is involved in contact-dependent T-cell activation of B-cells.

It can further be seen that the newly-submitted claims closely parallel claims recently issued to Lederman et al and assigned to Columbia University in U.S. Patent 5,993,816, on November 30, 1999. A copy of this patent is attached hereto for the convenience of the Examiner.

These claims should be allowable to Applicant for substantially the same reason that closely similar claims were allowed to Lederman et al. Also, the Examiner is respectfully advised that Lederman et al should not be applied as prior art under §102(e) because the effective filing date of this application is February 14, 1992, which is less than three months after November 15, 1991, the effective filing date of the Lederman '816

patent. Pursuant to §1.608, the undersigned respectfully asserts that there is a reasonable basis upon which the subject application should be entitled to an Interference judgment relative to the Lederman patent. Accordingly, a §131 Declaration is necessary.

Based thereon, Applicant hereby requests than an Interference be declared between this application and the Lederman '816 patent. For the convenience of the Examiner, the information required by §§1.607 and 1.608 is set forth under headings which correspond to the specific sections of §1.607 and §1.608.

(1) Identification of the Patent

Applicant seeks to provoke an interference between this application, having an effective filing date of February 14, 1992, and the Lederman patent, U.S. Patent No. 5,993,816, issued on November 30, 1999, having an effective filing date of November 15, 1991.

The claims of the Lederman patent are directed to use of a monoclonal antibodies which specifically bind to the antigen to which a particular monoclonal antibody, 5c8, specifically binds, and labeled forms thereof, to inhibit humoral immunity, immunoglobulin production, and B-cell activation, *in vitro* and *in vivo*.

(2) Suggestion of Proposed Count

Applicant hereby proposes the following Count to define the interfering subject matter. The proposed Count is an alternative Count prepared after careful consideration of the subject matter claimed by the respective parties.

An alternative Count is proposed because the present application and the Lederman patent define the same invention in different ways. More particularly, as described below, the patentable invention of the Count relates to use of monoclonal antibody which specifically bind an antigen selectively expressed on activated (not resting) T cells, which is involved in B cell activation, to inhibit humoral immunity immunoglobulin protection and B cell activation.

Specifically, in the Lederman patent this antigen is referred to by various names, i.e., "T-B cell activating molecule", "T-BAM", "CD40 ligand" (see column 2, lines 17-20) and the "5c8 antigen" based on its reactivity with a monoclonal antibody produced by a specific deposited hybridoma cell disclosed in the patent.

For example, at Col. 2, lines 17-20, the Lederman patent states that their

> "invention provides a monoclonal antibody which specifically recognizes and forms a complex with T-B cell activating molecule (T-BAM) (also know (SIC) as CD40 ligand) a protein located on the surface of activated T cells and thereby inhibits T cell activation of B cells."

The subject application similarly claims use of a monoclonal antibody which reacts with the same T cell antigen as the 5c8 monoclonal antibody of Lederman. However, in the subject application this same antigen is referred to by different names. Specifically, in the subject application, this antigen is referred to alternatively as the "CD40 counter receptor", "CD40CR", or is defined based on its reactivity with a specific monoclonal antibody produced by a deposited cell line, "MR1".

It is further noted that the 5c8 monoclonal antibody in the Lederman patent and the MR1 antibody of the subject application respectively specifically bind the human and murine counterparts of the same antigen expressed on activated T cells, which antigen is the counter receptor for CD40 expressed by B cells.

The fact that the MR1 antibody of the subject application binds the same antigen as the 5c8 antibody was conceded by the Patentees during prosecution in earlier Lederman patent 5,474,771 were involved in Interference. For example, the Patentees asserted at page 6 of their May 23, 1994 Response, the following:

> "MR1 is a hamster anti-T-BAM antibody that is similar with respect to mAb 5c8 with respect to its ability to inhibit contact dependent activation of B cells in vitro"...[i]t is therefore anticipated that both 5c8 and MR1 would have similar biological activities in humans and mice respectively".

Moreover, the Patentees stated "an antibody raised against a non-human T-BAM, such as MR1 which recognizes mouse T-BAM, is the best available animal model and would be accepted in the art".

The Patentees further stated at page 7 of this same Reply the following:

> "In a murine model of human autoimmune disease, anti-murine - T-BAM mAb MR1 was shown to inhibit collagen-induced antibodies."

Further at page 8, lines 8-9, the Patentees referred to the "therapeutic utility of an analogous anti-T-BAM mAb."

Thus, based on the Patentees' own admissions, it is clear that the subject application and the Lederman patent are both directed to use of monoclonal antibodies which bind the same T cell antigen to inhibit humoral immunity, immunoglobulin production, or B-cell activation. Similar to the parent application, which is involved in an earlier Interference with another Lederman patent (5,474,771) Applicant notes that the proposed Count has been drafted so as to encompass methods of using of monoclonal antibodies which bind either the murine or human counterpart of this T cell antigen, as these monoclonal antibodies, based on Patentee's own admissions, would be expected to possess "analogous" function and be obvious over one another.

The proposed Count is as follows:

> A method for inhibiting a humoral immune response, immunoglobulin production, and/or B-cell activation by contacting T-cells with an antibody that specifically binds to a protein specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession Number HB 10916; or
>
> A method for inhibiting a humoral immune response, immunoglobulin production, and/or B-cell activation by contacting T-cells with an antibody that specifically binds to the protein specifically recognized by monoclonal antibody MR1 produced by the hybridoma having ATCC Accession Number HB 11048; or
>
> A method for inhibiting a humoral immune response, immunoglobulin production, and/or B-cell activation by contacting T-cells with an antibody that specifically binds to CD40CR.

(3) Identification of Patented Claims Corresponding to the Proposed Count

Claims 1-14 of the Lederman patent correspond to the proposed Count. These claims read as follows:

1. A method of inhibiting a humoral immune response, comprising contacting T cells with an antibody that binds specifically to a protein specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession Number HB 10916.

2. A method of inhibiting immunoglobulin production, comprising contacting T cells with an antibody that specifically binds to a protein specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession Number HB 10916.

3. A method of inhibiting activation of B cells, comprising contacting T cells with an antibody that specifically binds to a protein specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession Number HB 10916.

4. A method of inhibiting a humoral immune response in an animal comprising the step of administering to the animal, in an amount effective to inhibit the humoral immune response, an antibody that binds specifically to a protein specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession Number HB 10916.

5. A method of inhibiting immunoglobulin production in an animal, comprising the step of administering to the animal, in an amount effective to inhibit the immunoglobulin production, an antibody that binds specifically to a protein specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession Number HB 10916.

6. A method of inhibiting activation of B cells in an animal, comprising the step of administering to the animal, in an amount effective to inhibit activation of B cells, an antibody that binds specifically to a

protein specifically recognized by monoclonal antibody 5c8 produced by the hybridoma having ATCC Accession Number HB 10916.

7. The method of claims 1, 2, 3, 4, 5, or 6, wherein the antibody is selected from the group consisting of: monoclonal antibodies, chimeric antibodies, human antibodies and humanized antibodies.

8. The method of claim 7, wherein the antibody 5c8 is produced by the hybridoma having ATCC Accession Number HB 10916.

9. The method of claim 7, wherein the antibody is conjugated to a therapeutic agent.

10. The method of claim 9, wherein the therapeutic agent is selected from the group consisting of: radioisotopes, toxins, toxoids and chemotherapeutic agents.

11. The method of claim 3 or 6, wherein the B cells are selected from the group consisting of: resting B cells and primed B cells.

12. The method of claim 4, 5 or 6, wherein the antibody is a chimeric monoclonal antibody, a humanized monoclonal antibody, a murine monoclonal antibody or a human monoclonal antibody.

13. The method of claim 4, 5 or 6, wherein the animal is a mammal.

14. The method of claim 13, wherein the mammal is a human.

It should be noted that Applicant is not conceding the validity of Claims 1-14, but are merely noting that they contain subject matter corresponding to the proposed Count. Essentially, all of the patent claims are directed to methods of using antibodies having the same binding specificity as the 5c8 antibody for inhibiting humoral immunity, immunoglobulin production, or B cell activation which, based on Patentees' own

admissions, bind the same antigen as MR1 (human counterpart) and would be expected to comprise analogous function. Applicant further notes that in accordance with 37 C.F.R. §1.606, the proposed Count corresponds to the broadest claims of the Lederman patent.

In particular, as discussed above, Claims 1-6 of the Lederman patent correspond identically to the first alternative of the proposed Count.

Dependent claims 7-14 similarly are directed to the same patentable invention as the Count. Claim 7 merely provides that the anti-5c8 antibody is a monoclonal, chimeric, human or humanized antibody which would have been obvious over the Count as these were well known types of antibodies at the time of the Lederman invention.

Claims 8 and 12 are directed to use of the monoclonal antibody produced by the deposited cell line HB 10916, which should be held unpatentable over the Count absent any unexpected results relative to the genus.

Claims 9 and 10 provide that the monoclonal antibody is conjugated to a therapeutic agent which should be held unpatentable over the Count since conjugation of effector (therapeutic) moieties to antibodies was well known as of the effective filing date of the Columbia patent.

Claim 11 provides that the administered antibody inhibits resting or primed B cells which should be held unpatentable over the Count as this is obvious over the Count which provides that the antibody inhibits B cell activation.

Claims 13 and 14 are unpatentable over the Count as they merely provide that humoral immunity, immunoglobulin production, or B cell activation is effected in a mammal or human, which would be obvious over the Count, based on the fact that *in vivo* suppression of humoral immunity, B cell activation, and immunoglobulin production, e.g., for treating allergies, and other antibody-related diseases, in mammals, and especially humans, was well known as of the effective filing date of the Columbia patent.

Thus, based on the foregoing, all of the claims of the Lederman patent correspond to the proposed Count.

(4) Requirements of 35 U.S.C. § 135(b) are Satisfied

The subject application and claims are being submitted less than one year after the issue date of the Lederman patent. Thus, the requirements of 35 U.S.C. §135(b) are satisfied.

Also, it is respectfully requested that this application be accorded special status as required under 37 C.F.R. §1.607(6)(b), because of the present Request to provoke an interference with an issued patent.

Further, according to §1.608(6)(d), Applicant understands that the Patentees will be given notice of the present Request to provoke an interference.

(5) *Prima Facie* Showing Required to Provoke an Interference between an Application and a Patent

The effective filing date of this application is February 14, 1992, which is less than three months after November 15, 1991, which is the effective filing date of the Lederman patent. The undersigned hereby asserts that there is a reasonable basis upon which the subject application should be entitled to a judgment relative to the Patentees.

(6) Identification of Claims in the Subject Application Corresponding to the Proposed Count

All of Claims 42-58 correspond to the proposed Count. In particular, all of these claims corresponds to the second or third alternative of the proposed Count. As required by 37 CFR §1.607(5)(ii), because these claims are newly-submitted, the basis for these claims in the as-filed application is set forth above.

As in the Lederman patent, Applicant's claims are directed to methods of using an antibody wherein said monoclonal antibody specifically binds an antigen expressed on activated T cells, and further wherein said antigen is the same antigen specifically bound by the MR1 antibody (which antigen is also referred to in the subject application as "CD40CR"), to inhibit humoral immunity, immunoglobulin production, or B-cell activation.

CONCLUSION

Based on the foregoing, Applicant respectfully submits that a prompt declaration of Interference between this application and U.S. Patent No. 5,993,816, to Lederman et al, is in order and such action is therefore earnestly solicited.

Also, the Examiner is respectfully advised that Judge Torczon requested in an Order in the earlier Interference between the parties (Interference No. 104,415) that this continuation application be filed and taken up specially by the Examiner so that it could potentially be included in the earlier Interference. For the Examiner's convenience, a copy of the Order is attached to this paper.

If the Examiner has any questions concerning any aspect of this matter, he is respectfully requested to contact the undersigned. In particular, if there are any problems concerning this Request, Applicant would like to schedule a personal interview with the Examiner to expedite prosecution.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: _____

Robin L. Teskin
Registration No. 35,030

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Date: December 20, 1999

5624-175
RB #44055-41X

THE CD40CR RECEPTOR AND LIGANDS THEREFOR

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THE CD40CR RECEPTOR AND LIGANDS THEREFOR

1. INTRODUCTION

The present invention relates to a counter-receptor, termed CD40CR, for the CD40 B-cell antigen, and to soluble ligands for this receptor, including fusion molecules comprising at least a portion of CD40 protein. It is based, at least in part, on the discovery that a soluble CD40/immunoglobulin fusion protein was able to inhibit helper T-cell mediated B-cell activation by binding to a novel 39 kD protein receptor on helper T-cell membranes. The present invention provides for a substantially purified CD40CR receptor; for soluble ligands of CD40CR, including antibodies as well as fusion molecules comprising at least a portion of CD40 protein; and for methods of controlling B-cell activation which may be especially useful in the treatment of allergy or autoimmune disease.

2. BACKGROUND OF THE INVENTION

Studies by Mitchison, Benacerraf and Raff first suggested that physical interactions between T_h and B-cells were essential in the development of humoral immune responses. Later studies documented that T_h formed physical conjugates with class II major histocompatibility complex (MHC) compatible, antigen-presenting B-cells *(Vitetta et al., (1987) Immunol. Rev. 99:193-239)* and that it was the B-cells within these conjugates that responded to T_h *(Bartlett et al., (1989) J. Immunol. 143:1745-1754)*. With the discovery that T_h-derived lymphokines exerted potent growth and differentiative effects on B-cells, it was proposed that soluble factor(s) released in proximity by activated T_h mediated the activation of the interacting

B-cell. However, none of the molecularly cloned lymphokines, alone or in combination, manifested the ability to induce B-cell cycle entry. Unlike soluble factors, plasma membrane fractions from activated T_h induced B-cell cycle entry *(Hodgkin et al., (1990) J. Immunol. 145:2025-2034; Noelle et al., (1991) J. Immunol. 146:1118-1124)*. Studies using purified plasma membrane fractions from activated T_h suggested that a protein expressed on the membrane of activated T_h was responsible for initiating humoral immunity *(Noelle et al., (1991) J. Immunol. 146:1118-1124; Bartlett et al., (1990) J. Immunol. 145:3956-3962)*.

Purified plasma membranes from activated T_h (PM^{Act}) have been used to investigate the nature of this effector function *(Hodgkin et al. (1990) J. Immunol. 145:2025-2034; Noelle et al., (1991) J. Immunol. 146:1118-1124)*. PM^{Act} from activated T_h, but not resting T_h (PM^{rest}) expressed an activity that induced B-cell cycle entry in an antigen-nonspecific, class II-unrestricted manner. In addition, it was shown that the activity expressed by PM^{Act} required 4-6 hours of activation, *de novo* RNA synthesis and was protein in nature *(Bartlett et al., (1990) J. Immunol. 145:3956-3962)*.

SUMMARY OF THE INVENTION

The present invention relates to a counter-receptor, termed CD40CR, for the CD40 B-cell antigen, and to soluble ligands for this receptor, including fusion molecules comprising at least a portion of CD40 protein. It is based, at least in part, on the discovery that a soluble CD40/immunoglobulin fusion protein was able to inhibit helper T-cell mediated B-cell activation by binding to a novel 39 kD receptor protein (termed "CD40CR" for CD40 counter-receptor) on

helper T-cell membranes, and on the discovery that a monoclonal antibody, termed MR1, directed toward this 39 kD receptor was able to inhibit helper T-cell mediated activation of B-cells.

The present invention provides for a substantially purified CD40CR receptor; for soluble ligands of CD40CR, including antibodies as well as fusion molecules comprising at least a portion of CD40 protein; and for methods of controlling B-cell activation.

In particular embodiments of the invention, B-cell activation in a subject may be inhibited by contacting helper T cells of the subject with effective amounts of a soluble ligand of CD40CR. Such inhibition of B-cell activation may be especially useful in the treatment of allergy or autoimmune disease.

One advantage of the present invention is that it enables intervention in an aspect of the immune response which is not antigen specific. Many current therapies for allergy include desensitization to particular antigens, and require that each patient be tested in order to identify antigens associated with sensitivity. As a practical matter, exhaustive analysis of a patient's response to each and every potential allergen is virtually impossible. Furthermore, in most autoimmune conditions, the causative antigen is, generally, unknown or even irrelevant to the disease process. The present invention, which relates to the antigen nonspecific CD40/CD40CR interaction, circumvents the need to characterize the antigen associated with allergy or autoimmunity. Therefore, the present invention may be used to particular advantage in the treatment of allergic conditions in which the immunogen is not

known, or has multiple components, for example, in hay fever or in procainamide induced lupus. It may also be useful in acute treatment of immune activation, for example, in therapy for anaphylaxis.

3.1. ABBREVIATIONS

Ig	immunoglobulin
mab	monoclonal antibody
PM^{Act}	plasma membranes prepared from activated helper T-cells
PM^{rest}	plasma membranes prepared from resting helper T-cells
PAGE	polyacrylamide gel electrophoresis
rIL4	recombinant interleukin 4
rIL5	recombinant interleukin 5
SN	supernatant
T_h	helper T-cell
T_h1	refers to D 1.6, a I-A^d-restricted, rabbit immunoglobulin specific clone

4. DESCRIPTION OF THE FIGURES

Figure 1. Effect of monoclonal antibodies and CD40-Ig on the induction of B-cell RNA synthesis by PM^{Act}.

Panel A. Resting B-cells were cultured with PM^{rest} or PM^{Act} from T_h1. 25μg/ml of anti-CD4, anti-LFA-1 or anti-ICAM-1 or a combination of each of these (each at 25 μg/ml) was added to wells containing PM^{Act} and B-cell RNA synthesis was measured by incorporation of [^{3}H]-uridine. B-cell RNA synthesis was assessed from 42 to 48 hours post-culture. Results presented are the arithmetic means of triplicate cultures +/- s.d., and are representative of 5 such experiments.

Panel B. Resting B-cells were cultured with PM^{Act} from T_h1 (●, ▲) or T_h2 (□). To the T_h1 PM^{Act}

containing cultures (●, ▲), increasing amounts of CD40-Ig (▲) or control protein CD7E-Ig(◆) were added. To the T_h2 PM^{Act} containing culture (□), increasing amounts of CD40-Ig were added. B-cell RNA synthesis was assessed from 42 to 48 hours post-culture. Results presented are the arithmetic means of triplicate cultures +/- s.d., and are representative of 3 such experiments.

Panel C. Resting B-cells were cultured with LPS (50 μg/ml) or PM^{Act}. To cultures, CD40-Ig (25 μg/ml; hatched) or CD7E-Ig (25 μg/ml; solid) were added. RNA synthesis was determined as described in Panel A. Results presented are the arithmetic mean of triplicate cultures +/- s.d., and are representative of 3 such experiments.

Figure 2. CD40-Ig inhibited B-cell differentiation and proliferation.

Panel A. Resting B-cells were cultured with PM^{Act}, rIL4 (10 ng/ml) and rIL5 (5 ng/ml). Either at the initiation of culture, or on days 1, 2 or 3 post-initiation of culture, CD40-Ig or CD7E-Ig (25 μg/ml) were added. On day six of culture, SN from individual wells were harvested and quantitated for IgM(■) and IgG_1(●) using an anti-isotype specific ELISA, as described in (*Noelle et al., (1991) J. Immunol. 146:1118-1124*). In the presence of PM^{Act}, IL4 and IL5, (in the absence of added CD40-Ig) the concentrations of IgM and IgG_1 were 4.6 μg/ml and 126 ng/ml, respectively. Cultures which received CD7E-Ig (25 μg/ml) on Day 0 produced 2.4 μg/ml and 89 ng/ml of IgM and IgG_1, respectively. In the absence of IL4 and IL5, no IgM or IgG_1 was detected. Results are representative of 3 such experiments.

Panel B. T_h1 were rested or activated with anti-CD3 for 16 hours, irradiated and cultured ($1x10^4$/well) with resting B-cells ($4x10^4$/culture) in the presence of IL4 (10 ng/ml). Between 0 and 25 μg/ml of CD40-Ig (▲) or CD7E-Ig (●) were added to cultures. From 66-72 hours post-culture, wells were pulsed with 1.0 μCi of [^{3}H]-thymidine and harvested. The dotted line indicates the response of B-cells to resting T_h. Results presented are the arithmetic mean of triplicate cultures +/- s.d., and are representative of 2 such experiments.

Figure 3. CD40-Ig detected a molecule expressed on activated, but not resting T_h. Resting and activated T_h were harvested and incubated with fusion proteins for 20 minutes at 4°C, followed by FITC-conjugated goat anti-hIgG (25 μg/ml). Percentage positive cells and MFI were determined by analysis of at least 5000 cells/sample. Results are representative of 6 such experiments. CD40-Ig binding is indicated by a filled-in profile.

Figure 4. CD40-Ig immunoprecipitated a 39 kD protein from lysate of activated T_h1. T_h1 were rested or activated with insolubilized anti-CD3 for 16 hours. [^{35}S]-labelled proteins from resting or activated T_h were immunoprecipitated with purified antibodies or fusion proteins (1-10μ). The gel profile is representative of 3 such experiments.

Figure 5. A monoclonal antibody (mab), specific to the induced 39 Kd T_h membrane protein, inhibited induction of B-cell RNA synthesis by PM^{Act}. Resting B-cells and PM^{Act} were cultured with 10 μg/ml each of anti-α/β, anti-CD3, CD40-Ig or MR1. RNA synthesis was

determined as described in Figure 1. Results presented are the arithmetic means of triplicate cultures +/- s.d., and are representative of 3 such experiments.

Figure 6. MR1 and CD40-Ig recognized the same molecule expressed on activated T_h.

Panel A: Activated T_h were fluorescently stained with MR1 or control Ig. To evaluate if CD40-Ig and MR1 competed for binding to activated T_h, graded concentrations of MR1 or control hamster Ig (anti-α/β TCR) were added together with anti-CD40 (20 μg/ml). After incubation for 20 minutes at 4°C, the samples were washed and incubated with FITC-conjugated, mab anti-human IgG_1. Results are representative of 3 such experiments.

Panel B: Proteins from [^{35}S]-methionine-labelled, activated T_h were immunoprecipitated with MR1 (10 μg/sample) or CD40-Ig (10 μg/sample) and resolved by PAGE and fluorography. Results presented are representative of 2 such experiments.

Figure 7. Binding of CD40-Ig to human cell lines. A variety of human T-cell lines were exposed to biotin-labelled CD40-Ig, and binding was evaluated by flow cytometry.

Figure 8.

Panel A: Nucleotide sequence of CD40 cDNA from *Stamenkovic et al., (1989) EMBO J. 8:1403-1410.* The transmembrane region is underscored.

Panel B: Schematic diagram of a plasmid that may be used to express CD40-Ig. The amino acid sequences at the site of fusion of Δ CD40 is shown below the diagrammed portion of CD40.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for substantially purified CD40CR receptor; for soluble ligands of CD40CR, including antibodies as well as fusion molecules comprising CD40; and for methods of controlling B-cell activation.

For purposes of clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the following subsections:

(i) ligands that bind to CD40CR;
(ii) methods used to characterize CD40CR;
(iii) preparation of purified CD40CR;
(iv) uses of ligands that bind to CD40CR; and
(v) uses of CD40CR.

5.1. LIGANDS THAT BIND TO CD40CR

The present invention provides for soluble ligands of CD40CR, including (i) fusion molecules comprising at least a portion of CD40 protein and (ii) antibodies or antibody fragments.

The term "soluble," as used herein, indicates that the ligands of the invention are not permanently associated with a cell plasma membrane. Soluble ligands of the invention may, however, be affixed to a non-cellular solid support, including a lipid, protein, or carbohydrate molecule, a bead, a vesicle, a magnetic particle, a fiber, etc. or may be enclosed within an implant or vesicle.

The ability of such a ligand to bind to CD40CR may be confirmed by demonstrating that the ligand binds to the same protein as CD40-Ig (infra) or MR1 (infra).

The ligands of the invention may be comprised in pharmaceutical compositions together with a suitable carrier.

5.1.1. FUSION MOLECULES

The present invention provides for soluble fusion molecules that are ligands of CD40CR. Such fusion molecules comprise at least a portion of CD40 protein attached to a second molecule. The portion of CD40 preferably lacks the CD40 transmembrane domain. A portion of CD40 protein which may be used according to the invention is defined as any portion which is able to bind to CD40CR, for example, such a portion may be shown to bind to the same protein as MR1 or CD40-Ig.

Second molecules which may be used include peptides and proteins, lipids, and carbohydrates, and, in preferred embodiments of the invention, may be an immunoglobulin molecule, or portion thereof (such as an Fv, Fab, $F(ab')_2$, or Fab' fragment) or CD8, or another adhesion molecule, such as B7. The second molecule may be derived from either a non-human or a human source, or may be chimeric. The second molecule may also be an enzyme, toxin, growth factor, lymphokine, antiproliferative agent, alkylating agent, antimetabolite, antibiotic, vinca alkaloid, platinum coordinated complex, radioisotope, or a fluorescent compound.

The fusion molecules of the invention may be produced by chemical synthesis or, preferably, by recombinant DNA techniques.

For example, a nucleic acid sequence encoding at least a portion of CD40 protein may be combined with a nucleic acid sequence encoding a second molecule in a suitable expression vector, and then expressed in a prokaryotic or, preferably, eukaryotic expression

system, such as a yeast, baculovirus, or mammalian expression system, including transgenic animals.

Alternatively, at least a portion of CD40 protein may be expressed using recombinant DNA techniques and then may be chemically conjugated to a second molecule.

Fusion molecules comprising CD40 may be purified from preparative mixtures using electrophoretic techniques or affinity chromatography using ligand that binds to either CD40 or to the second molecule. Ligands that bind to CD40 include, but are not limited to, anti-CD40 antibodies such as G28-5, as produced by the hybridoma having accession number HB9110 and deposited with the American Type Culture Collection, and CD40CR, described more fully in sections 5.2 and 5.3, infra. If the second molecule is an immunoglobulin or immunoglobulin fragment, an affinity column comprising anti-immunoglobulin antibody may be used; if the second molecule comprises an F_c fragment, a protein A column may be used.

According to a preferred embodiment of the invention, a portion of CD40 may be produced using a nucleic acid sequence that encodes a CD40 protein that is truncated upstream from the transmembrane domain. Such a nucleic acid sequence may be prepared by digesting a plasmid containing a cDNA encoding CD40 antigen, such as that described in *Stamenkovic et al., (1989), EMBO J. 8:1403-1410*, with PstI (P) and Sau 3A (S3) restriction enzymes. The resulting P/S3 fragment may be subcloned into the same plasmid digested with P and Bam HI (B), to produce a truncated CD40 gene (see Figure 8).

In particular, nonlimiting, embodiments of the invention, an expression vector used to produce ligands containing at least a portion of CD40 as well

as immunoglobulin sequence may preferably comprise a virally-derived origin of replication, a bacterial origin of replication, a bacterial selectable marker, and eukaryotic promoter and enhancer sequences separated from DNA sequences encoding an immunoglobulin constant region by restriction endonuclease sites which allow subcloning of DNA sequenes encoding at least a portion of CD40, followed by a polyadenylation signal sequence (see Figure 8.b.).

In a specific embodiment of the invention, the truncated CD40 gene may be subcloned into an immunoglobulin fusion plasmid, such as that described in *Aruffo et al., 1990, Cell 61:1303-1313*, using an Mlu I and B digest, to form plasmid pCD40-Ig, which encodes the fusion molecule CD40-Ig (see Figure 8). CD40-Ig fusion protein may then be produced by transfecting the pCD40-Ig plasmid into COS cells to form a transient expression system. CD40-Ig produced may be collected from the COS cell supernatant and purified by protein A column chromatography as described in *Aruffo et al., 1990, Cell 161:1303-1313*.

5.1.2. ANTIBODIES

The soluble ligands of the invention may comprise antibody molecules, monoclonal antibody molecules, or fragments of these antibody molecules which contain an antigen combining site that binds to CD40CR. Such ligands may further comprise a second molecule which may be a protein, lipid, carbohydrate, enzyme, toxin, growth factor, lymphokine, antiproliferative agent, alkylating agent, antimetabolite, antibiotic, vinca alkaloid, platinum coordinated complex, radioisotope, or a fluorescent compound and may be linked to the antibody molecule or fragment.

Where the ligand is a monoclonal antibody, or a fragment thereof, the monoclonal antibody can be prepared against CD40CR using any technique which provides for the production of antibody molecules by continuous cell lines in culture. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497) as well as other techniques which have more recently become available, such as the human B-cell hybridoma technique (Kozbar et al., 1983, Immunology Today 4:72) and EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96) and the like are within the scope of the present invention.

Antibody fragments which contain the idiotype of the molecule could be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment which can be generated by treating the antibody molecule with pepsin; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragment; the $F(ab')_2$ fragment which can be generated by treating the antibody molecule with papain; and the 2Fab or Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent to reduce the disulfide bridges.

The present invention also provides for chimeric antibodies produced by techniques known in the art, such as those set forth in *Morrison et al., (1984) Proc. Natl. Acad. Sci. U.S.A. 81:6851-6855* or *European Patent Application No. 85305604.2, publication No. 0173494 by Morrison et al., published March 5, 1986.*

Immunogen for the production of antibodies may be any source that contains CD40CR. For example, activated T_h may be used as an immunogen.

Alternatively, substantially purified CD40CR, prepared as set forth in section 5.3, infra, may be used. If activated T_h are used as immunogen, antiserum may be tested for reactivity against activated but not resting T_h cells.

In a preferred embodiment of the invention, the soluble ligand is the MR1 monoclonal antibody. The following method was used to produce the MR1 monoclonal antibody, and may be used to generate other antibodies directed toward CD40CR.

Hamsters were immunized intraperitoneally with $5\text{-}10^6$ activated T_h1 cells (D1.6) at weekly intervals for six weeks. When the serum titer against murine T_h1 was greater than about 1:10,000, cell fusions were performed with polyethylene glycol using immune hamster splenocytes and NSI. SN from wells containing growing hybridomas were screened by flow cytometry on resting and activated T_h1. One particular hybridoma, which produced a mab that selectively recognized activated T_h, was further tested and subcloned to derive MR1. MR1 was produced in ascites and purified by ion exchange HPLC.

The present invention also provides for ligands comprising monoclonal antibodies, and fragments thereof that are capable of competitively inhibiting the binding of MR1 to its target antigen or CD40-Ig to its receptor.

5.2. METHODS USED TO CHARACTERIZE CD40CR

CD40CR may be characterized by (i) its ability to bind CD40, fusion molecules comprising at least a portion of CD40, and antibodies such as MR1; (ii) its functional characteristic of being able to stimulate B-cell cycle entry, proliferation, and differentiation; and (iii) its cellular distribution.

5.2.1. ABILITY TO BIND LIGANDS

CD40CR may be characterized by its ability to bind to ligands such as CD40, fusion molecules comprising CD40, and antibodies directed toward CD40CR.

As discussed in greater detail infra, several techniques were used to characterize CD40CR. For example, CD40-Ig and MR1 were shown to recognize the same 39 kD molecule. Both CD40-Ig and MR1 were found to immunoprecipitate a 39 kD protein from radiolabelled T_h lysates (Figure 5b). Further, immunoprecipitation of the 39 kD protein with CD40-Ig removed the antigen recognized by MR1 from T_h lysates.

5.2.2. ABILITY TO STIMULATE B-CELLS

CD40CR may also be characterized by its ability to stimulate B-cell cycle entry, proliferation, and differentiation.

For example, plasma membrane (PM) from activated (PM^{Act}) but not resting (PM^{rest}) T_h cells was found to induce B-cell RNA synthesis (Figure 1a); this induction, indicative of B-cell activation, was not affected by antibodies such as anti-LFA-1, anti-CD4, anti-ICAM-1. CD40-Ig or MR1, however, were found to be able to inhibit PM^{Act} -induced B-cell activation, as shown in Figure 1b and Figure 6.

The induction of B-cell activation may be measured by techniques such as [^{3}H]-uridine incorporation into RNA (as B-cells differentiate, RNA synthesis increases), or by [^{3}H]-thymidine incorporation, which measures DNA synthesis associated with cell proliferation. For optimal measurement of the effect of CD40CR on B-cell proliferation, interleukin-4 (IL-4) may be added to the culture medium at a concentration of about 10 ng/ml.

Alternatively, B-cell activation may be measured as a function of immunoglobulin secretion. For example, CD40CR, in substantially purified form, or as present in PM, or otherwise, may be added to resting B-cells together with IL-4 (10 ng/ml) and IL-5 (5 ng/ml). After three days of culture, an additional volume of culture medium may be added. On day 6 of culture, supernatant (SN) from individual cultures may be harvested and quantitated for IgM and IG_1 as described in *Noelle et al., (1991) J. Immunol. 146:1118-1124.*

5.2.3. CELLULAR DISTRIBUTION

CD40CR may also be characterized by its cellular distribution. For example, CD40-Ig was observed to bind to activated, but not resting T_h1, as assessed by flow cytometry (Figure 3). Furthermore, CD40-Ig was observed to bind to Jurkat cells, HSB2 cells, and activated T-cells from human peripheral blood, but did not appear to bind significantly to CEM cells, HPBALL cells, or murine thymoma cells.

For example, and not by way of limitation, the presence of CD40CR on a particular cell type ("test cells") may be evaluated by flow cytometry as follows. Test cells may be tested in parallel with resting (negative control) and activated (positive control) T_h cells. All cells may be incubated at a concentration of about 1 x 10^5 cells/50 μl with ligand (e.g. CD40-Ig or MR1) for 20 minutes at 4°C, followed by FITC-conjugated anti-ligand antibody. Propidium iodide may be added to all samples to a final concentration of 2 μg/ml. Flow cytometric analysis may then be performed, for example on a BD FACSCAN. After positive gating of cells by forward versus side scatter, and by red negativity (for propidium iodide

exclusion), and the log green fluorescence of viable cells may be ascertained.

5.3. PREPARATION OF PURIFIED CD40CR

The present invention provides for substantially purified CD40CR. Such CD40CR may be prepared from cells bearing CD40CR, such as activated helper T-cells, Jurkat, and HSB2 cells, by the following method.

Plasma membranes may be prepared from appropriate cells, such as activated T_h1 cells, by discontinuous sucrose gradient sedimentation, as described in *Noelle et al., 1991, J. Immunol.* 146:1118-1124. CD40CR may then be isolated by dissociating the crude membrane extract with mild detergent, and then performing size exclusion chromatography followed by either affinity chromatography using appropriate ligands (e.g. MR1 or CD40-Ig) bound to a solid support, immunoprecipitation (e.g. by CD40-Ig or MR1), and/or gel electrophoresis.

The resulting protein may be expected to have a molecular weight of about 39 kD.

The present invention provides for a soluble CD40CR (i.e. cell-free) which may be comprised in pharmaceutical compositions together with a suitable carrier. It further provides for CD40CR which is linked to a second molecule which may be a peptide, protein, lipid, carbohydrate, enzyme, toxin, growth factor, lymphokine, antiproliferative agent, alkylating agent, antimetabolite, antibiotic, vinca alkaloid, platinum coordinated complex, radioisotope, or a fluorescent compound.

The present invention further provides for substantially purified CD40CR which has been prepared by chemical synthesis or recombinant DNA techniques. For example, the gene for CD40CR may be isolated by inserting cDNA prepared from activated helper T-cells

into the λgt10 expression system, and then screening with MR1 or CD40-Ig binding to identify CD40CR-expressing clones. Alternatively, cDNA prepared from activated helper T-cells may be transfected into COS cells, the supernatants of which may be screened with MR1 or CD40-Ig to identify CD40CR producers. The gene for CD40CR may be then used to express CD40CR using expression systems known in the art.

5.4. USES OF LIGANDS THAT BIND TO CD40CR

The present invention provides for methods of controlling B-cell activation that utilize ligands that bind to CD40CR. In particular, it provides for a method of inhibiting B-cell activation comprising exposing a mixture of B-cells and T_h cells to an effective concentration of ligand that binds to CD40CR. Ligands that may be used are described supra in section 5.1. The method of the invention may be practiced in vitro or in vivo. An effective concentration refers to a concentration of a ligand that inhibits B-cell activation, measured by any technique known in the art (including those set forth in section 5.2, supra) by at least about 30 percent, and preferably by about 75 percent. According to a preferred, specific, non-limiting embodiment of the invention, CD40-Ig may be used as ligand, in which case an effective concentration may be at least about 10 μg/ml. In another specific, nonlimiting embodiment of the invention, the monoclonal antibody MR1 may be used, in which case an effective concentration may be at least about 10 μg/ml. If the method is practiced in vivo, an effective concentration of ligand may refer to plasma concentration of ligand or to a local concentration. For example, it may be desirable to

inhibit B-cell activation in a localized area in order to limit the effects on the immune system as a whole.

In particular embodiments, the invention provides for a method of treating a subject suffering from a disorder associated with B-cell activation, comprising administering to the subject a therapeutic amount of ligand that binds to CD40CR. A subject may be a non-human or, preferably, a human animal.

Disorders associated with B-cell activation include, but are not limited to, allergy (including anaphylaxis); autoimmune conditions including drug induced lupus, systemic lupus erythematosus, adult rheumatoid arthritis, juvenile rheumatoid arthritis, scleroderma, Sjogren's Syndrome, etc.; and viral diseases that involve B-cells, including Epstein-Barr infection, and retroviral infection including infection with a human immunodeficiency virus.

Because it has been suggested that B-cell activation is associated with the induction of human immunodeficiency virus replication from latency, it may be desirable to administer the ligands of the invention to HIV positive individuals who have not yet developed AIDS or ARC.

Ligands may be administered, in a suitable pharmaceutical carrier, by any method known in the art, including intravenous, intraperitoneal, subcutaneous, intrathecal, intraarticular or intramuscular injection, and oral, intranasal, intraocular and rectal administration, and may be comprised in microspheres, liposomes, and/or sustained release implants.

A therapeutic amount of ligand is defined as an amount which significantly diminishes the deleterious clinical effects of B-cell activation, and may vary among ligands used and conditions treated. If CD40-Ig

is used, therapeutic concentration may be about 10 μg/ml either systemically (plasma concentration) or locally. If MR1 is used, a therapeutic concentration may be about 10 μg/ml either systemically (plasma concentration) or locally.

In a further embodiment of the invention, the above methods may utilize a ligand comprising a toxin or antimetabolite such that T_h cells are killed or damaged and B-cell activation is decreased as a result of T_h cell destruction.

The ligands of the invention may also be used to label activated T cells, a technique which may be useful in the diagnosis of T cell disorders. To this end, ligand comprising an enzyme, radioisotope, fluorescent compound or other detectable label may be exposed to T cells in vitro or in vivo and the amount of binding may be quantitated.

The ligands of the invention may also be used to deliver substances, e.g. growth factors, to activated T-cells.

5.5. USES OF CD40CR

The present invention provides for methods of controlling B-cell activation that utilize CD40CR or a molecule comprising CD40CR, prepared as described in section 5.3, supra. In particular, it provides for a method of promoting B-cell activation comprising exposing B-cells to an effective concentration of CD40CR. The method may be practiced in vivo or in vitro. An effective concentration refers to a concentration of receptor that induces B-cell activation, measured by any technique known in the art (including those set forth in section 5.3, supra) by at least about 30 percent. In specific, nonlimiting

embodiments of the invention, the concentration of CD40CR may be about 10 μg/ml locally or systemically.

In particular embodiments, the invention provides for a method of treating a subject suffering from an immunodeficiency disorder associated with diminished humoral immunity, comprising administering to the subject a therapeutic amount of CD40CR. A subject may be a non-human or, preferably, a human animal.

Immunodeficiency disorders associated with diminished humoral immunity include acquired immunodeficiency syndrome, immunodeficiency associated with malignancy or cachexia, iatrogenic immunodeficiency caused, for example, by chemotherapy or radiation therapy, as well as genetid disorders involving humoral immunity.

CD40CR may be administered, in a suitable pharmaceutical carrier, by any method known in the art, including intravenous, intraperitoneal, subcutaneous, intrathecal, intraarticular, or intramuscular injection, and oral, intranasal, intraocular, and rectal administration and may be comprised in microspheres, liposomes, and/or sustained release implants.

A therapeutic amount of CD40CR for CD40 is defined as that amount which increases immunoglobulin production by at least about 30 percent.

In a further embodiment, CD40CR may be conjugated to a toxin, and then administered to a subject under circumstances in which it would be preferable to destroy B-cells that express CD40. Examples of such circumstances include patients receiving organ transplants or suffering from multiple myeloma or another B-cell malignancy, or from autoimmune disease.

CD40CR may also be used to label B-cells expressing CD40, a technique which may be useful in

the diagnosis of B-cell disorders. To this end, receptor linked to an enzyme, radioisotope, fluorescent compound or other detectable label may be exposed to B-cells *in vivo* or *in vitro* and the amount of binding may be quantitated.

CD40CR may also be used to deliver molecules that are linked to it to B-cells.

6. EXAMPLE; A NOVEL RECEPTOR, CD40CR, ON ACTIVATED HELPER T-CELLS BINDS CD40 AND TRANSDUCES THE SIGNAL FOR COGNATE ACTIVATION OF B-CELLS

6.1. MATERIALS AND METHODS

6.1.1. ANIMALS

Female DBA/2J mice (Jackson Laboratories, Bar Harbor, ME) were used for the preparation of filler cells to support the growth of T_h clones and in the preparation of resting B-cells.

6.1.2. HELPER T-CELL CLONES (T_h)

D1.6, a I-A^d-restricted, rabbit Ig-specific T_h1 clone *(Kurt-Jones et al., (1987) J Exp Med 166:1774-1787)* was obtained from Dr. David Parker, University of Mass. at Worcester. D1.6 will be referred to herein as T_h1.

6.1.3. ACTIVATION OF T_h BY ANTI-CD3

T_h1 were cultured (8 x10^6/well) in cluster wells (6 well, Corning, NY) coated with 40 µg/4 ml of PBS/well with anti-CD3 for 16 hours, as described in *(Noelle et al., (1991) J. Immunol. 146:1118-1124)*.

6.1.4. PREPARATION OF T_h PLASMA MEMBRANES

Plasma membranes were prepared by discontinuous sucrose gradient sedimentation, as described in *(Noelle et al., (1991) J. Immunol. 146:1118-1124)*.

6.1.5. PREPARATION OF RESTING B-CELLS

Resting splenic B-cells were prepared by sedimentation on discontinuous Percoll gradients, as described in *(Defranco et al., (1982) J. Exp. Med. 155:1523)*. Cells isolated from the 70-75% (density of 1.087-1.097) Percoll interface were typically >95% mIg^+, had a uniform, low degree of near forward light scatter and were unresponsive to Con A.

6.1.6. ANTIBODIES

The following mabs were purified by ion exchange HPLC from ascites fluid of mice which had been irradiated and bone marrow reconstituted: anti-CD3:145-2C11 *(Leo et al., (1987) Proc. Natl. Acad. Sci. USA 84:1374-1378)*; anti-α,β:H57-597; anti-CD4: GK1.5 *(Wilde et al., (1983) J. Immunol. 131:2178-2183)*; anti-ICAM:YN1/1.7.4 *(Prieto et al., (1989) Eur. J. Immunol. 19:1551-1557)*; anti-LFA-1: Fb441.8 *(Sarmiento et al, (1982) Immunol. Rev. 68:135)*; and anti-rat/hamster κ chain:RG-7 *(Springer, (1982) Hybrid. 1:257-273)*.

6.1.7. PREPARATION OF THE CD40 RECOMBINANT GLOBULIN (CD40-Ig)

The CD40 fusion protein was prepared by digesting a plasmid containing a cDNA encoding the CD40 antigen *(Stamenkovic and Seed, (1989) EMBO J. 8:1403-1410)* with the restriction enzyme Pst I (P) and Sau 3A (S3). This P/S3 fragment was subcloned into the same plasmid digested with P and Bam H1 (B). This allowed the preparation of the CD40Δ which encoded a CD40 protein truncated upstream from the transmembrane domain. The DNA fragment encoding a CD40Δ was then subcloned into the immunoglobulin fusion plasmid *(Aruffo et al, (1990), Cell. 61:1303-1313)* using a MluI and B digest. The CD40-Ig fusion protein was produced by transient

transfection in COS cells and purified on a protein A column as described in ((*Aruffo et al., (1990) Cell. 61:1303-1313*).

6.1.8. LYMPHOKINES

Interleukin 4 (IL4): Recombinant mouse IL4 was generously provided by Drs. C. Maliszewski and K. Grabstein, Immunex Corporation, Seattle, WA.

Interleukin 5 (IL5): Recombinant mouse IL5 was purchased from R&D Research, Sarrento, CA.

6.1.9. INDUCTION OF B-CELL RNA SYNTHESIS BY ACTIVATED T_h PLASMA MEMBRANES

3 X 10^4 resting B-cells were cultured in 50 μl of cRPMI in A/2 microtiter wells (Costar, Cambridge, MA). To these wells, 0.5 μg of T_h1 or T_h2 membrane protein was added. From 42-48 hrs, wells were pulsed with 2,5 μCi of ^{3}H-uridine (New England Nuclear, Boston MA), harvested, and the radioactivity determined by liquid scintillation spectroscopy. The results were expressed as cpm/culture +/-s.d.

6.1.10. INDUCTION OF B-CELL IMMUNOGLOBULIN SECRETION BY ACTIVATED T_h PLASMA MEMBRANES AND LYMPHOKINES

Resting B-cells were cultured as described above. To culture wells, 0.5 μg of T_h1 membrane protein, IL4 (10 ng/ml) and IL5 (5 ng/ml) were added. On day three of culture, an additional 50 μl of cRPMI was added. On day six of culture, SN from individual wells were harvested and quantitated for IgM and IgG_1, as described in *(Noelle et al., (1991) J. Immunol. 146:1118-1124)*.

6.1.11. INDUCTION OF B-CELL PROLIFERATION BY ACTIVATED T_h AND IL4

$4x10^4$ resting B-cells were cultured in 50 µl of cRPMI in A/2 microtiter wells (Costar, Cambridge, MA). To these wells, $1x10^4$ resting or activated, irradiated (500 rads) T_h1 and IL4 (10 ng/ml) were added. On day three of culture, wells were pulsed with 1 µCi of ^3H thymidine, as described in *(Noelle et al., (1991) J. Immunol. 146:1118-1124)*.

6.1.12. PRODUCTION OF MONOCLONAL ANTIBODIES SPECIFIC TO MEMBRANE PROTEINS INDUCED ON ACTIVATED T_h1

Hamsters were immunized intraperitoneally with 5-10 x 10^6 activated T_h1 (D1.6) at weekly intervals for six weeks. When the serum titer against murine T_h1 was greater than 1:10,000, cell fusions were performed with polyethylene glycol using immune hamster splenocytes and NS1. SN from wells containing growing hybridomas were screened by flow cytometry on resting and activated T_h1. One particular hybridoma, which produced a mab that selectively recognized activated T_h, was further tested and subcloned to derive MR1. MR1 was produced in ascites and purified by ion exchange HPLC.

6.1.13. FLOW CYTOFLUOROMETRIC ANALYSIS OF ACTIVATION MOLECULES EXPRESSED ON T_h

Resting and activated T_h(16 hours with anti-CD3) were harvested and incubated at $1x10^5$ cells/50 µl with fusion protein for 20 minutes at 4°C, followed by FITC-conjugated goat anti-human (h)IgG (25 µg/ml; Southern Biotechnology, Birmingham, AL). To all samples, propidium iodide was added at final concentration of 2 µg/ml. Flow cytofluorometric

analysis was performed on a BD FACSCAN. After positive gating of cells by forward versus side scatter, and by red negativity (for propidium iodide exclusion), the log green fluorescence of viable cells was ascertained. At least 5,000 viable cells were analyzed for the determination of percent positive cells and MFI. Staining with MR1 employed FITC-conjugated RG7, a mouse anti-rat/hamster κ chain mab.

6.1.14. BIOSYNTHETIC LABELLING, IMMUNOPRECIPITATION, SDS-PAGE AND FLUOROGRAPHY

T_h1 were rested or activated with insolubilized anti-CD3 for 16 hrs. Proteins from resting and activated T_h(20 x 10^6/ml) were labelled with 1 mCi of [^{35}S]-methionine/cysteine for one hour, at which time they were washed twice in RPMI/10%FCS and the cell pellet was lysed in extraction buffer,as described *(Noelle et al., (1986) J. Immunol. 137:1718-1726)*. Purified antibodies or fusion proteins (1-10 μg) were added to 500μl of lysate (5x10^6 cell equivalents) at 4°C for 16 hours. At that time, the lysates were transferred to tubes containing 50 μl of packed Protein A-sepharose. The pelleted Protein A-Sepharose was resuspended and tubes were incubated at 4°C for 1 hr with agitation. The samples were then washed 3x with high stringency wash buffer. The pelleted protein A-Sepharose was resuspended in 30 μl of SDS sample buffer and run on a 10% polyacrylamide gel. After running the gel, the gel was fixed and fluorography performed.

6.2. RESULTS

6.2.1. EFFECT OF MONOCLONAL ANTIBODIES ON THE INDUCTION OF B-CELL RNA SYNTHESIS BY PM^{Act}

In order to define the cell surface molecules that mediated the induction of B-cell cycle entry by PM^{Act}, mabs to T_h membrane proteins were added to cultures of PM^{Act} and B-cells. PM^{Act} induced B-cell RNA synthesis eight-fold over that observed with PM^{rest}) (Figure 1a). The addition of anti-LFA-1, anti-CD4, anti-ICAM-1, alone, or in combination, did not inhibit the induction of B-cell RNA synthesis by PM^{Act}.

6.2.2. CD40-Ig INHIBITED T_h-INDUCED B-CELL CYCLE ENTRY, DIFFERENTIATION AND PROLIFERATION

In the human system, it had been shown that anti-CD40 mab induced B-cell proliferation *(Clark and Lane, (1991) Ann. Rev. Immunol. 9:97-127)* thereby implicating CD40 as an important triggering molecule for B-cells. To determine if CD40 was involved in the induction of B-cell RNA synthesis by PM^{Act}, a soluble fusion protein of the extracellular domains of human CD40 and the F_c domain of human IgG_1 (CD40-Ig) was added to cultures of PM^{Act} and B-cells. PM^{Act} derived from T_h1 and T_h2 were prepared and used to stimulate B-cell RNA synthesis. The addition of CD40-Ig to culture caused a dose-dependent inhibition of B-cell RNA synthesis that was induced by PM^{Act} from T_h1 and T_h2 (Fig. 1b). Half-maximal inhibition of B-cell RNA synthesis induced by PM^{Act} from T_h1 and T_h2 was about 5 μg/ml CD40-Ig. A CD7E-Ig fusion protein *(Damle and Aruffo, (1991) Proc. Natl. Acad. Sci. USA 88:6403-6407)* was without effect even when used at 25 μg/ml.

To investigate whether CD40-Ig inhibited the activation of B-cells by T-independent activators, B-cells were cultured in the presence of LPS and CD40-Ig. On day 2, RNA synthesis was assessed (Fig. 1c). CD40-Ig was ineffective at inhibiting B-cell activation by LPS, yet inhibited the response of B-cells to PM^{Act}.

In the presence of PM^{Act}, IL4 and IL5, B-cells polyclonally differentiated to produce Ig (*Hodgkin et al., (1990) J. Immunol. 145:2025-2034; Noelle et al., (1991) J. Immunol. 146:1118-1124*). To evaluate the requirements for CD40 signalling in this process, CD40-Ig was added at the initiation of culture, or on subsequent days of culture. The addition of CD40-Ig (Fig. 2a) at the initiation of culture inhibited greater than 95% of polyclonal IgM and IgG_1 production compared to control levels in its absence. In contrast, the addition of CD40-Ig on day 1 and 2 of culture showed little, if any, inhibitory effect on IgM and IgG_1 production. These data indicated that after 24 hours, signalling via CD40 is no longer essential for the differentiation of B-cells to Ig secretion.

Data thus far indicated that CD40 was implicated in the activation of B-cells by PM^{Act}. Studies were performed in order to ensure that CD40 was also involved in the activation of B-cells by intact, viable, activated T_h. T_h1 were activated for 16 hours with insolubilized anti-CD3, harvested and irradiated. The irradiated T_h1 were cultured with B-cells in the presence of IL4 and B-cell proliferation was determined on day 3 of culture. An exogenous source of IL4 was required to achieve B-cell proliferation with T_h1, because T_h1 do not produce IL4 (*Noelle et al., (1989) J. Immunol. 143:1807-1814*). CD40-Ig

inhibited the induction of B-cell proliferation by irradiated T_h in a dose-dependent manner, similar to that observed with PM^{Act} (Fig. 2b). The negative control, CD7E-Ig, exerted no appreciable effect.

6.2.3. CD40-Ig DETECTED A MOLECULE EXPRESSED ON ACTIVATED, BUT NOT RESTING T_h

To investigate whether activated T_h1 express a binding protein for CD40, resting and activated (16 hours) T_h1 were stained with CD40-Ig or CD7E-Ig, followed by FITC-anti-HIgG. Binding of CD40-Ig was assessed by flow cytometry (Fig. 3). T_h1 that were activated for 16 hours with anti-CD3, but not resting T_h1, stained 56% positive with CD40-Ig, but not with the control CD7E-Ig. To identify the CD40-Ig binding protein, T_h1 proteins were biosynthetically labelled with [^{35}S]-methionine/cysteine and proteins immunoprecipitated with CD40-Ig or CD7E-Ig. The immunoprecipitated proteins were resolved by SDS-PAGE and fluorography (Figure 4). A prominent band with an apparent molecular weight of 39 kD immunoprecipitated in a dose-dependent manner with 1 and 10 μg of CD40/sample. As controls, anti-class I mab immunoprecipitated bands at 55 kD and a low molecular weight band, β2 microglobulin. In the absence of mab, no prominent bands were visible. A 39 kd band was also immunoprecipitated from activated T_h that were vectorially labelled with ^{125}I, confirming that the 39kD protein was a membrane protein.

6.2.4. MONOCLONAL ANTIBODY MR1, SPECIFIC TO 39Kd T_h MEMBRANE PROTEIN, INHIBITED THE INDUCTION OF B-CELL RNA SYNTHESIS BY PM^{Act}

Mabs specific to antigens selectively expressed on activated versus resting T_h were developed to

identify T_h molecule(s) responsible for the T_h effector phase activity. One such mab, MR1, recognized an antigen that was selectively expressed on activated T_h1. To investigate whether MR1 and CD40-Ig recognized the same molecule, flow cytometry and blocking studies were performed. CD40-Ig and MR1 stained approximately 56% and 61%, respectively, of activated, but not resting Th (Fig. 5a). MR1, but not another hamster anti-T cell mab, anti-α/β TCR, blocked the staining of activated T_h1 with CD40-Ig, in a dose-dependent manner. These data suggested that CD40-Ig and MR1 recognized overlapping or identical epitopes on the 39 kD Th protein. To further demonstrate that CD40-Ig and MR1 recognized the same molecule, the antigen that bound MR1 was identified by immunoprecipitation of proteins from radiolabelled Th lysates. Both CD40-Ig and MR1 immunoprecipitated a 39 kD protein (Fig. 5b). Finally, immunoprecipitation of the 39kD protein with CD40-Ig removed the antigen recognized by MR1 from radiolabelled lysates of activated T_h supporting the tenet that the MR1 antigen and the CD40 binding protein were identical.

Functional studies were performed with MR1 to address whether this mab neutralized the activity expressed by PM^{Act}. PM^{Act} and B-cells were cultured alone, or in the presence of hamster mabs or CD40-Ig. Two hamster mabs, anti-α/β TCR and α-CD3 did not inhibit the activation of resting B-cells by PM^{Act}. In contrast, MR1 or CD40-Ig inhibited B-cell activation (Fig. 6).

6.3. DISCUSSION

The data show that blocking of prominent T_h surface molecules (LFA-1, CD4, ICAM-1, CD3, α,β TCR) with mabs did not impede the capacity of activated T_h

to induce B-cell cycle entry. In contrast, CD40-Ig or a maB specific to the CD40 binding protein, blocked T_h-dependent B-cell activation in a dose-dependent manner. Furthermore, the CD40 binding protein was identified as a 39 kD protein that is selectively expressed on the membranes of activated, but not resting T_h. Both CD40-Ig and a mab specific to the 39kD CD40 binding protein blocked B-cell activation by PM^{Act}.

Although a number of membrane proteins have been implicated in T_h-dependent B-cell signalling, evidence presented herein dismisses the contribution of some molecules (LFA-1, CD4, CD3, α,β TCR, ICAM-1) and implicates CD40 as the B-cell receptor for cognate signalling by T_h. Data show that CD40-Ig and a mab specific to the CD40 binding protein inhibits T_h-dependent B-cell activation.

The ligand for CD40 is a 39Kd protein that is expressed on activated, but not resting T_h. Biochemical studies indicate that the 39kD protein is a single chain molecule since electrophoretic migration was not influenced by reducing agents. Based on the functional studies presented in this study, both activated T_h1 and T_h2 express the 39 kD CD40 binding protein. This is consistent with the functional studies that show both T_h1 and T_h2 induce B-cell cycle entry. In an attempt to further characterize the 39 kD protein, cDNA encoding CD proteins in the MW range of 39kD (CD 53, CD27 and CD69) were transiently transfected into COS cells and the cells were tested for CD40-Ig binding. None of the transfected COS cells expressed proteins that bound CD40-Ig. It is therefore suspected that the 39 kD protein is not one of these CD proteins.

The biochemical basis for signal transduction between T_h and B-cells has been elusive. The identification of CD40 as the signal transducing molecule for T cell help focusses attention on specific biochemical pathways known to be coupled to the CD40 molecule. CD40 is a member of the nerve growth factor receptor (NGFR) family by virtue of the presence of four cysteine-rich motifs in its extracellular region. Signaling through CD40 by mab has been shown *(Uckun et al., (1991) J. Biol. Chem. 266:17478-17485)* to involve the activation of tyrosine kinases resulting in the increased production of inositol trisphosphate and the activation of at least four distinct serine/threonine kinases. Based on information obtained from signaling through other members of the NGF receptor family, it is anticipated that interaction between activated T_h and B will result in many of the same biochemical processes.

7. EXAMPLE: BINDING OF CD40 Ig TO HUMAN T-CELL LINES

For immunofluorescence binding studies, CD40 Ig fusion protein was conjugated with biotin using biotin-succinimide (Sigma). Flow cytometry analysis was then performed by tow-step staining using phycoerythrin (PE)-strepavidin (Bectin-Dickinson) with a Coulter Epics C instrument. Representative results of screening multiple T cell lines is presented below. The Jurkat and HSB2 cell lines were found to bind specifically, whereas other T cell lines including CEM, HPBALL, and murine thymoma did not bind the CD40 Ig fusion protein (Fig. 7).

Various publications are cited herein which are hereby incorporated by reference in their entirety.

WHAT IS CLAIMED IS:

1. A substantially purified CD40CR receptor.

2. The substantially purified receptor of claim 1 which has a molecular weight of about 39 kilodaltons.

3. The substantially purified receptor of claim 1 which binds to CD40-Ig.

4. The substantially purified receptor of claim 1 which binds to the monoclonal antibody MR1.

5. A soluble ligand for CD40CR, comprising at least a portion of CD40 protein.

6. The soluble ligand of claim 5 which comprises a portion of CD40 protein which lacks a transmembrane domain.

7. The soluble ligand of claim 5 or 6 which further comprises at least a portion of an immunoglobulin molecule.

8. The soluble ligand of claim 5 which is CD40-Ig.

9. A soluble ligand for CD40CR, comprising at least a portion of an immunoglobulin molecule.

10. The soluble ligand of claim 9 in which the immunoglobulin molecule is capable of

competitively inhibiting the binding of CD40 to CD40CR.

11. The soluble ligand of claim 9 in which the immunoglobulin molecule is capable of competitively inhibiting the binding of monoclonal antibody MR1 to its target antigen.

12. The soluble ligand according to claim 9, 10 or 11 which further comprises a second molecule which is an antiproliferative agent.

13. The soluble ligand according to claim 9, 10 or 11 which further comprises a second molecule which is an alkylating agent.

14. The soluble ligand according to claim 9, 10 or 11 which further comprises a second molecule which is an antimetabolite.

15. The soluble ligand according to claim 9, 10 or 11 which further comprises a second molecule which is an antibiotic.

16. The soluble ligand according to claim 9, 10 or 11 which further comprises a second molecule which is a vinca alkaloid.

17. The soluble ligand according to claim 9, 10 or 11 which further comprises a second molecule which is an enzyme.

18. The soluble ligand according to claim 9, 10 or 11 which further comprises a second molecule which is a platinum coordinated complex.

19. The soluble ligand according to claim 9, 10 or 11 which further comprises a second molecule which is a radioisotope.

20. The soluble ligand according to claim 9, 10 or 11 which further comprises a second molecule which is a fluorescent compound.

21. Monoclonal antibody MR1 or a fragment thereof.

22. A method of inhibiting B-cell activation comprising exposing a mixture of B-cells and helper T cells to an effective concentration of ligand that binds to CD40CR.

23. The method of claim 22 in which the ligand comprises at least a portion of CD40 protein.

24. The method of claim 22 in which the ligand comprises a portion of CD40 protein which lacks a transmembrane domain.

25. The method of claim 23 or 24 in which the ligand further comprises at least a portion of an immunoglobulin molecule.

26. The method of claim 22 in which the ligand is CD40-Ig.

27. The method of claim 22 in which the ligand comprises at least a portion of an immunoglobulin molecule.

28. The method of claim 27 in which the immunoglobulin molecule is capable of competitively inhibiting the binding of CD40 to CD40CR.

29. The method of claim 27 in which the immunoglobulin molecule is capable of competitively inhibiting the binding of monoclonal antibody MR1 to its target antigen.

30. A method of treating a subject suffering from a disorder associated with B-cell activation, comprising administering to the subject a therapeutic amount of ligand that binds to CD40CR.

31. The method of claim 30 in which the disorder is an allergy.

32. The method of claim 30 in which the disorder is an autoimmune disease.

33. The method of claim 30 in which the ligand comprises at least a portion of CD40 protein.

34. The method of claim 30 in which the ligand comprises a portion of CD40 protein which lacks a transmembrane domain.

35. The method of claim 33 or 34 in which the ligand further comprises at least a portion of an immunoglobulin molecule.

36. The method of claim 30 in which the ligand is CD40-Ig.

37. The method of claim 30 in which the ligand comprises at least a portion of an immunoglobulin molecule.

38. The method of claim 37 in which the immunoglobulin molecule is capable of competitively inhibiting the binding of CD40 to CD40CR.

39. The method of claim 37 in which the immunoglobulin molecule is capable of competitively inhibiting the binding of monoclonal antibody MR1 to its target antigen.

40. A pharmaceutical composition comprising CD40-Ig in a suitable pharmaceutical carrier.

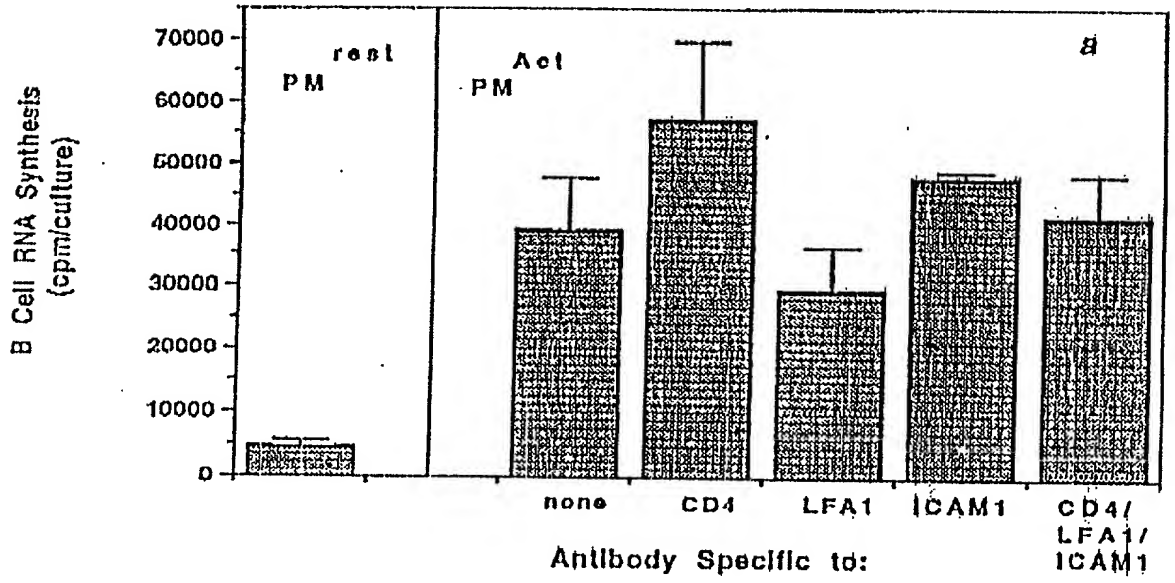
41. A pharmaceutical composition comprising monoclonal antibody MR1 or a fragment thereof in a suitable pharmaceutical carrier.

ABSTRACT

The present invention relates to a counter-receptor, termed CD40CR, for the CD40 B-cell antigen, and to soluble ligands for this receptor, including fusion molecules comprising at least a portion of CD40 protein. It is based, at least in part, on the discovery that a soluble CD40/immunoglobulin fusion protein was able to inhibit helper T-cell mediated B-cell activation by binding to a novel 39 kD protein receptor on helper T-cell membranes. The present invention provides for a substantially purified CD40CR receptor; for soluble ligands of CD40CR, including antibodies as well as fusion molecules comprising at least a portion of CD40 protein; and for methods of controlling B-cell activation which may be especially useful in the treatment of allergy or autoimmune disease.

5601-175 (sheet 1 of 9)

Figure 1.



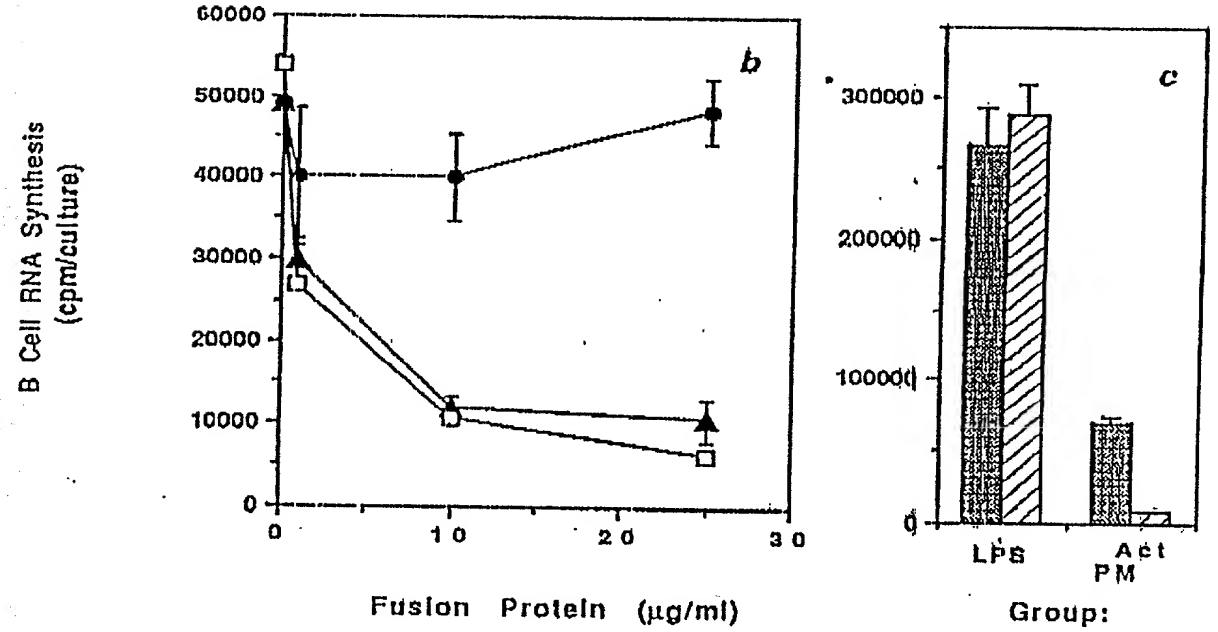


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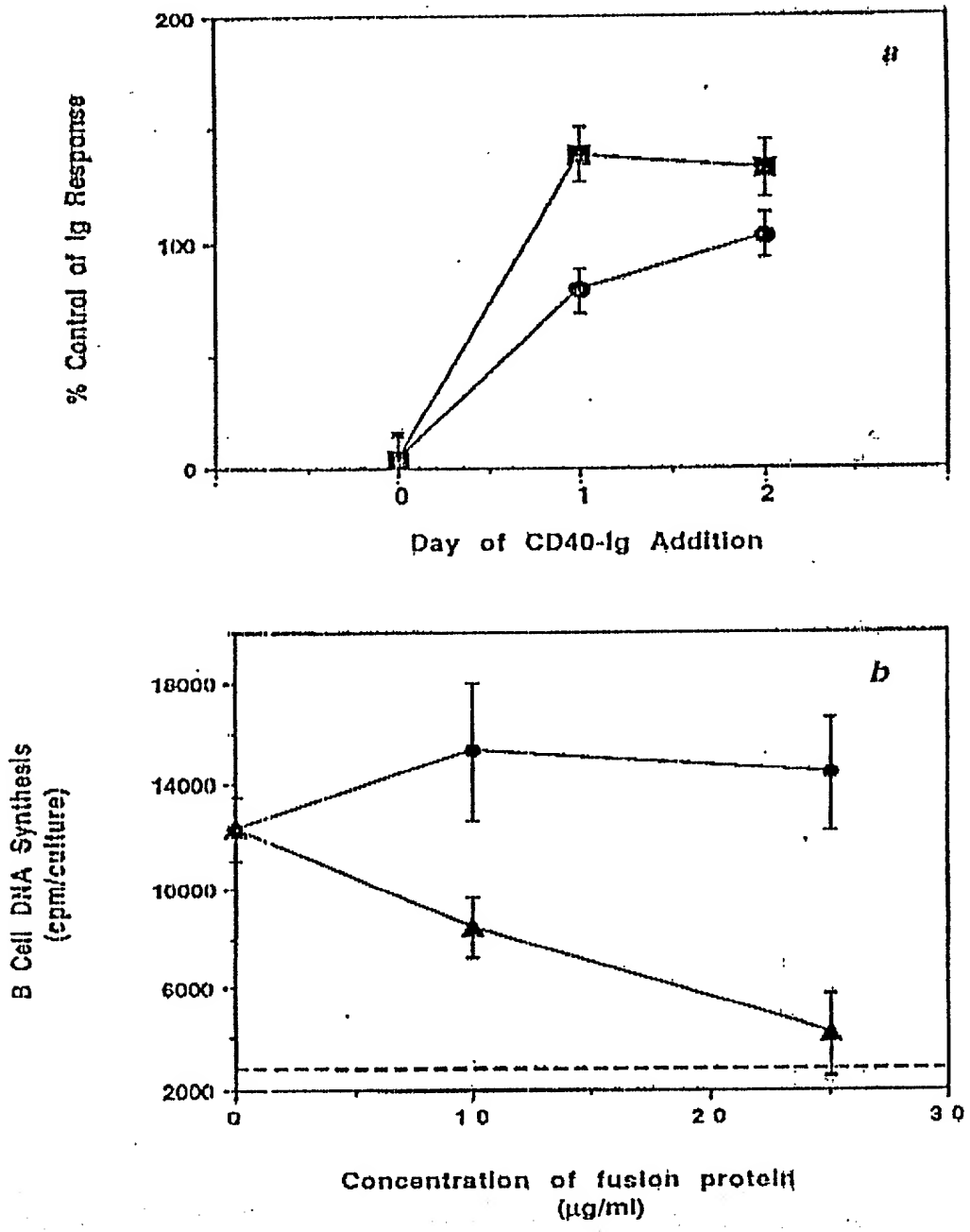
Figure 2.



5621-175 (sheet 3 of 9)

Figure 3.

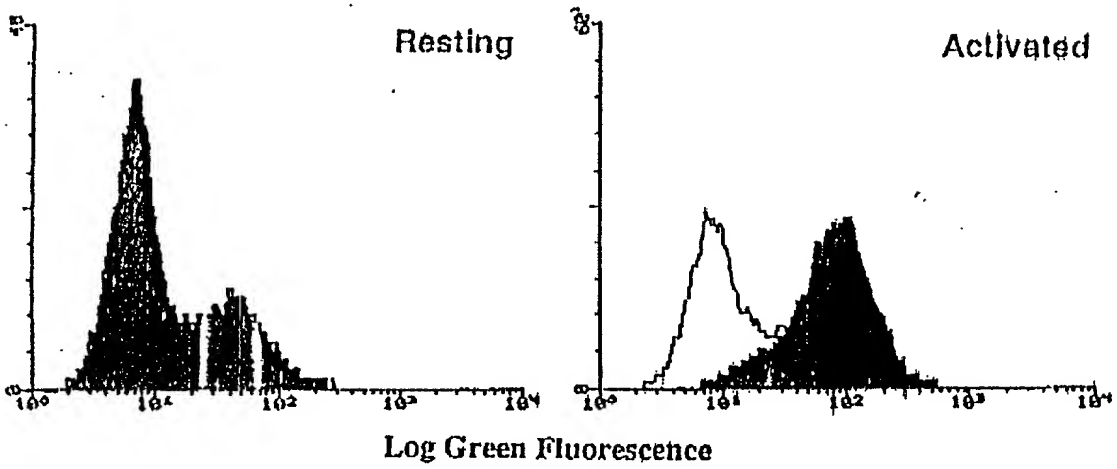


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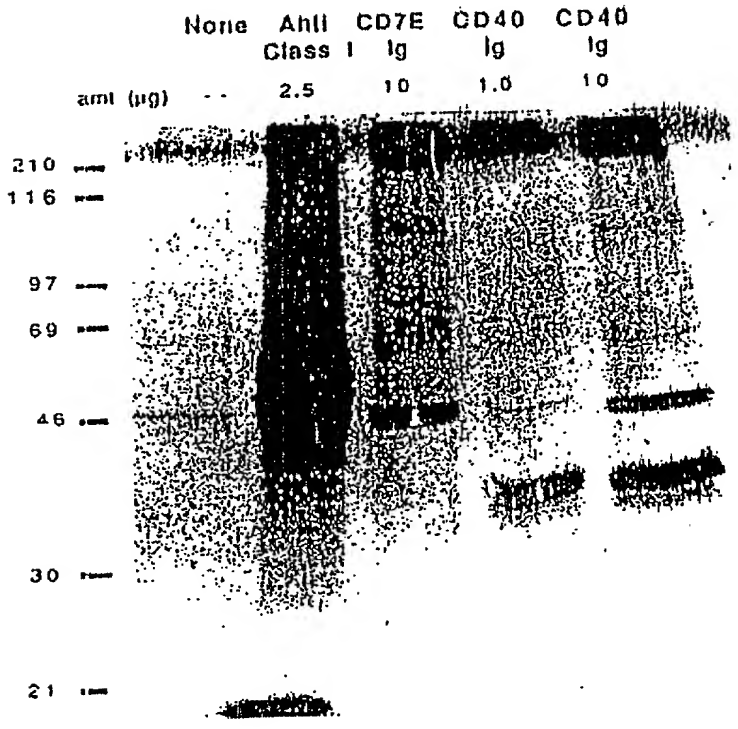
Figure 4.



562-175 (sheet 7 of 9)

Figure 5.

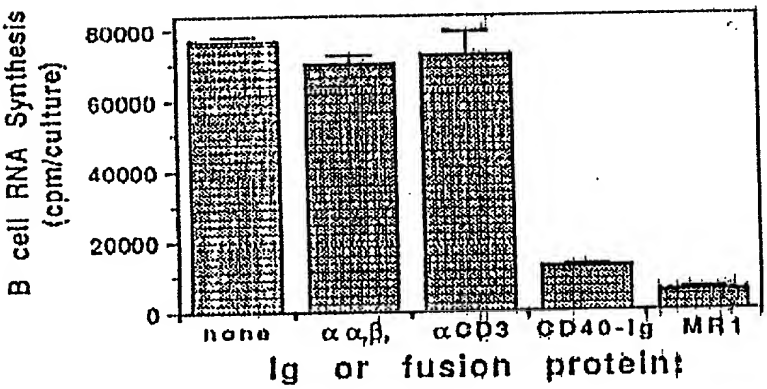


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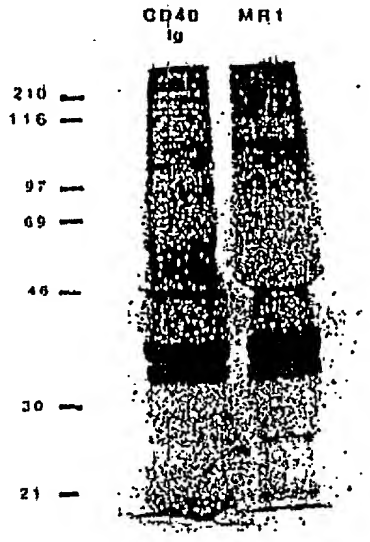
Figure 6.

a

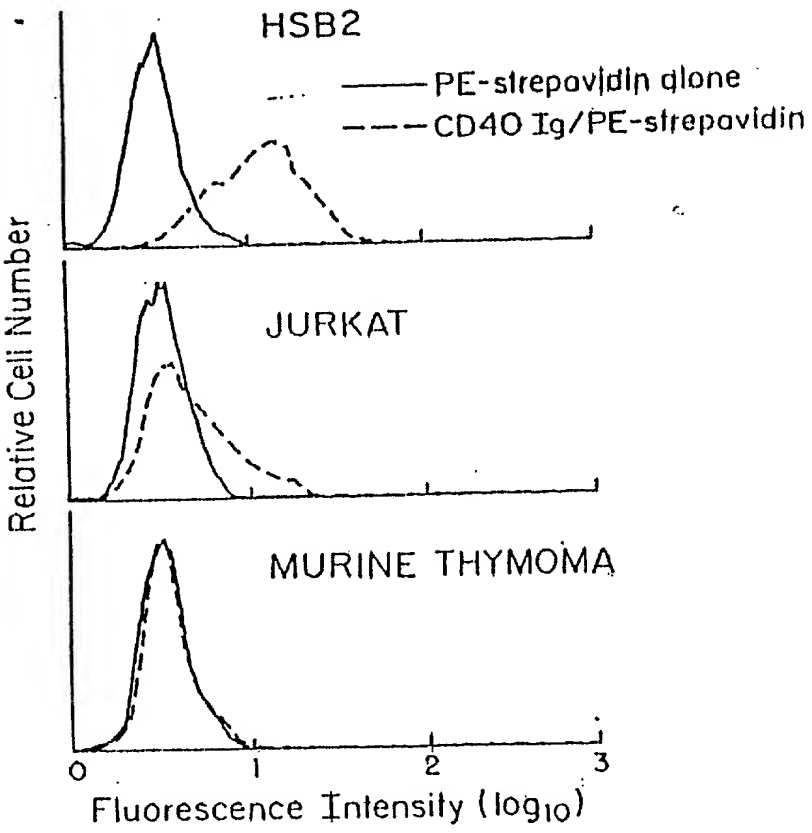
Staining Ab or Fusion Protein	Blocking Ab	Resting Th	Activated Th
CD40 (50 µg/ml)	---	4.9	38.5
MR1 (50 µg/ml)	---	5.7	9.8
CD40 (50 µg/ml)	MR1 (50 µg/ml)	5.0	11.7
CD40 " "	MR1 (25 µg/ml)	---	20.7
CD40 " "	MR1 (10 µg/ml)	---	30.3
CD40 " "	MR1 (5 µg/ml)	---	49.5
CD40 " "	α-α,β (50 µg/ml)	---	63.0

b



560-1-175 (sheet 7 of 9)

Figure 7.



56=1-175 (Sheet 2 of 4)

Figure 8A.

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  1                                                  M  V  R  L  P  L  Q  C  V  L  W  G  C  L  L  T  A  V  H  P  E  P  P  T  A

121 CATGCAGAGAAAAACAGTACCTAATAAACAGTCAGTGCTGTTCTTTGTGCCAGCCAGGACAGAAACTCGTGAGTGACTGCACAGAGTTCACTGAAACGGAATGCCTTCCTTGCGGTCAAA
 26  C  R  E  K  Q  Y  L  I  N  S  Q  C  C  S  L  C  Q  P  G  Q  K  L  V  S  D  C  T  E  F  T  E  T  E  C  L  P  C  G  E  S

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 66  E  F  L  D  T  W  N  R  E  T  H  C  H  Q  H  K  Y  C  D  P  N  L  G  L  R  V  Q  Q  K  G  T  S  E  T  D  T  I  C  T  C

361 GTGAAGAAGGCTGGCACTGTACGAGTGAGGCCTGTGAGAGCTGTGTCCTGCACCGCTCATGCTCGCCCGGCTTTGGGGTCAAGCAGATTGCTACAGGGGTTTCTGATACCATCTGCGAGC
106  E  E  G  W  H  C  T  S  E  A  C  E  S  C  V  L  H  R  S  C  S  P  G  F  G  V  K  Q  I  A  T  G  V  S  D  T  I  C  E  P

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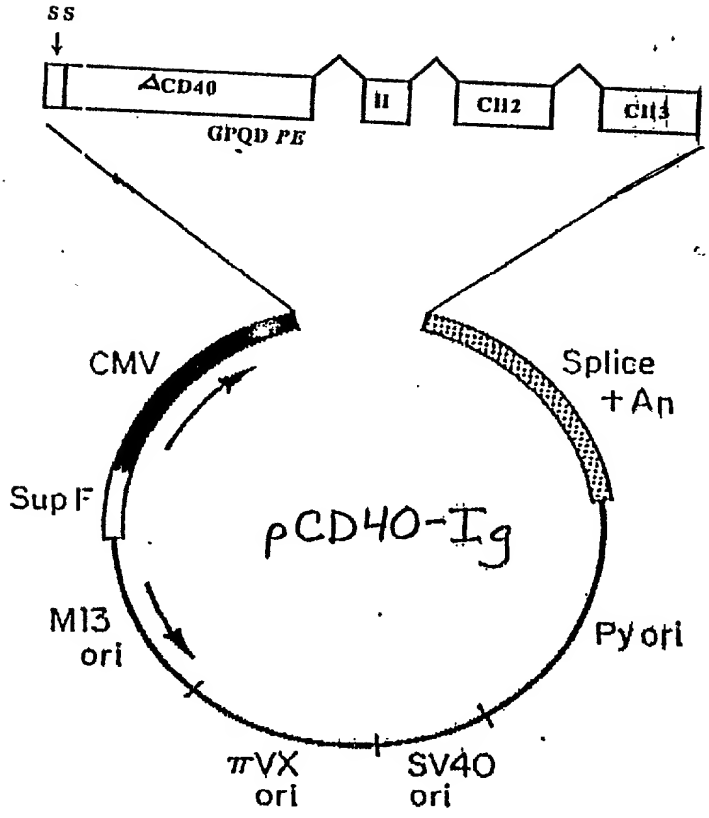
Figure 8B.



PENNIE & EDMONDS DOCKET NO. 5624-175

SUPPLEMENTAL DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below at 201 et seq. underneath my name.

I believe I am the original, first and sole inventor if only one name is listed at 201 below, or an original, first and joint inventor if plural names are listed at 201 et seq. below, of the subject matter which is claimed and for which a patent is sought on the invention entitled

THE CD40CR RECEPTOR AND LIGANDS THEREFOR

the specification of which:

is attached hereto
☒ was filed in the United States on February 14, 1992 as Application Serial No. 07/835,799 *(for declaration not accompanying application)*
with amendment(s) filed on _____ *(if applicable)*

☐ was filed as PCT international application Serial No. _____ on _____ and was amended under PCT Article 19 on _____ *(if applicable)*

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119/§172 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED PRIOR TO THE FILING DATE OF THE APPLICATION			
APPLICATION NUMBER	COUNTRY	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. 119/172
			YES ☐ NO ☐
			YES ☐ NO ☐
			YES ☐ NO ☐
			YES ☐ NO ☐

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS		
		PATENTED	PENDING	ABANDONED

POWER OF ATTORNEY: As a named inventor, I hereby appoint S. Leslie Misrock (Reg. No. 18872), Harry C. Jones, III (Reg. No. 20280), Berj A. Terzian (Reg. No. 20060), Gerald J. Flintoft (Reg. No. 20823), David Weild, III (Reg. No. 21094), Jonathan A. Marshall (Reg. No. 24614), Joseph V. Colaianni (Reg. No. 20019), Charles E. McKenney (Reg. No. 22795), Philip T. Shannon (Reg. No. 24278), Barry D. Rein (Reg. No. 22411), Stanton T. Lawrence, III (Reg. No. 25736), Francis E. Morris (Reg. No. 24615), Charles E. Miller (Reg. No. 24576), Gidon D. Stern (Reg. No. 27469), John J. Lauter, Jr. (Reg. No. 27814), Brian M. Poissant (Reg. No. 28462), Brian D. Coggio (Reg. No. 27624), Rory J. Radding (Reg. No. 28749), Stephen J. Harbulak (Reg. No. 29166), Donald J. Goodell (Reg. No. 19766), James N. Palik (Reg. No. 25510), Thomas E. Friebel (Reg. No. 29258), Laura A. Coruzzi (Reg. No. 30742), Jennifer Gordon (Reg. No. 30753) and Jon R. Stark (Reg. No. 30111), whose address is Pennie & Edmonds, 1155 Avenue of the Americas, New York, New York 10036, and each of them, my attorneys, to prosecute this application, and to transact all business in the Patent and Trademark Office connected therewith.

PENNIE & EDMONDS DOCKET NO. 5624-175

SEND CORRESPONDENCE TO:	PENNIE & EDMONDS 1155 AVENUE OF THE AMERICAS NEW YORK, N.Y. 10036-2711	DIRECT TELEPHONE CALLS TO: PENNIE & EDMONDS (212) 790-9090

201	FULL NAME OF INVENTOR	LAST NAME ARUFFO	FIRST NAME ALEJANDRO	MIDDLE NAME A.	
	RESIDENCE & CITIZENSHIP	CITY Edmonds	STATE OR FOREIGN COUNTRY Washington	COUNTRY OF CITIZENSHIP MEXICO/ITALY	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS 1012 Spruce Street	CITY Edmonds	STATE OR COUNTRY Washington	ZIP CODE 98020
202	FULL NAME OF INVENTOR	LAST NAME LEDBETTER	FIRST NAME JEFFREY	MIDDLE NAME A.	
	RESIDENCE & CITIZENSHIP	CITY Seattle	STATE OR FOREIGN COUNTRY Washington	COUNTRY OF CITIZENSHIP U.S.A.	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS 306 N.W. 113th Place	CITY Seattle	STATE OR COUNTRY Washington	ZIP CODE 98177
203	FULL NAME OF INVENTOR	LAST NAME STAMENKOVIC	FIRST NAME IVAN	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY Brookline	STATE OR FOREIGN COUNTRY Massachusetts	COUNTRY OF CITIZENSHIP SWITZERLAND	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS 59 Babcock Street	CITY Brookline	STATE OR COUNTRY Massachusetts	ZIP CODE 02146
204	FULL NAME OF INVENTOR	LAST NAME NOELLE	FIRST NAME RANDOLPH	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY Plainfield	STATE OR FOREIGN COUNTRY New Hampshire	COUNTRY OF CITIZENSHIP U.S.A.	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS Freeman Hill Road	CITY Plainfield	STATE OR COUNTRY New Hampshire	ZIP CODE 03781
205	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY	ZIP CODE
206	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY	ZIP CODE

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201 Alejandro A. Aruffo	SIGNATURE OF INVENTOR 202 Jeffrey A. Ledbetter	SIGNATURE OF INVENTOR 203 Ivan Stamenkovic
DATE 3/20 1992	DATE 3/20 1992	DATE 1992
SIGNATURE OF INVENTOR 204 Randolph Noelle	SIGNATURE OF INVENTOR 205	SIGNATURE OF INVENTOR 206
DATE 1992	DATE	DATE

PENNIE & EDMONDS DOCKET NO. 5624-175

SUPPLEMENTAL DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below at 201 et seq. underneath my name.

I believe I am the original, first and sole inventor if only one name is listed at 201 below, or an original, first and joint inventor if plural names are listed at 201 et seq. below, of the subject matter which is claimed and for which a patent is sought on the invention entitled

THE CD40CR RECEPTOR AND LIGANDS THEREFOR

the specification of which:

is attached hereto
☒ was filed in the United States on February 14, 1992 as Application Serial No. 07/835,799 *(for declaration not accompanying application)*
with amendment(s) filed on _____ *(if applicable)*

☐ was filed as PCT international application Serial No. _____ on _____ and was amended under PCT Article 19 on _____ *(if applicable)*

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119/§172 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED PRIOR TO THE FILING DATE OF THE APPLICATION			
APPLICATION NUMBER	COUNTRY	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. 119/172
			YES ☐ NO ☐
			YES ☐ NO ☐
			YES ☐ NO ☐
			YES ☐ NO ☐

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS		
		PATENTED	PENDING	ABANDONED

POWER OF ATTORNEY: As a named inventor, I hereby appoint S. Leslie Misrock (Reg. No. 18872), Harry C. Jones, III (Reg. No. 20280), Berj A. Terzian (Reg. No. 20060), Gerald J. Flintoft (Reg. No. 20823), David Weild, III (Reg. No. 21094), Jonathan A. Marshall (Reg. No. 24614), Joseph V. Colaianni (Reg. No. 20019), Charles E. McKenney (Reg. No. 22795), Philip T. Shannon (Reg. No. 24278), Barry D. Rein (Reg. No. 22411), Stanton T. Lawrence, III (Reg. No. 25736), Francis E. Morris (Reg. No. 24615), Charles E. Miller (Reg. No. 24576), Gidon D. Stern (Reg. No. 27469), John J. Lauter, Jr. (Reg. No. 27814), Brian M. Poissant (Reg. No. 28462), Brian D. Coggio (Reg. No. 27624), Rory J. Radding (Reg. No. 28749), Stephen J. Harbulak (Reg. No. 29166), Donald J. Goodell (Reg. No. 19766), James N. Palik (Reg. No. 25510), Thomas E. Friebel (Reg. No. 29258), Laura A. Coruzzi (Reg. No. 30742), Jennifer Gordon (Reg. No. 30753) and Jon R. Stark (Reg. No. 30111), whose address is Pennie & Edmonds, 1155 Avenue of the Americas, New York, New York 10036, and each of them, my attorneys, to prosecute this application, and to transact all business in the Patent and Trademark Office connected therewith.

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201	FULL NAME OF INVENTOR	LAST NAME ARUFFO	FIRST NAME ALEJANDRO	MIDDLE NAME A.	
	RESIDENCE & CITIZENSHIP	CITY Edmonds	STATE OR FOREIGN COUNTRY Washington	COUNTRY OF CITIZENSHIP MEXICO/ITALY	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS 1012 Spruce Street	CITY Edmonds	STATE OR COUNTRY Washington	ZIP CODE 98020
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203	FULL NAME OF INVENTOR	LAST NAME STAMENKOVIC	FIRST NAME IVAN	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY Brookline	STATE OR FOREIGN COUNTRY Massachusetts	COUNTRY OF CITIZENSHIP SWITZERLAND	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS 59 Babcock Street	CITY Brookline	STATE OR COUNTRY Massachusetts	ZIP CODE 02146
204	FULL NAME OF INVENTOR	LAST NAME NOELLE	FIRST NAME RANDOLPH	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY Plainfield	STATE OR FOREIGN COUNTRY New Hampshire	COUNTRY OF CITIZENSHIP U.S.A.	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS Freeman Hill Road	CITY Plainfield	STATE OR COUNTRY New Hampshire	ZIP CODE 03781
205	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY	ZIP CODE
206	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY	ZIP CODE

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SIGNATURE OF INVENTOR 201 Alejandro A. Aruffo	SIGNATURE OF INVENTOR 202 Jeffrey A. Ledbetter	SIGNATURE OF INVENTOR 203 Ivan Stamenkovic
DATE 1992	DATE 1992	DATE 1992
SIGNATURE OF INVENTOR 204 Randolph Noelle	SIGNATURE OF INVENTOR 205	SIGNATURE OF INVENTOR 206
DATE April 3 1992	DATE	DATE

PENNIE & EDMONDS DOCKET NO. 5624-175

SUPPLEMENTAL DECLARATION AND POWER OF ATTORNEY

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THE CD40CR RECEPTOR AND LIGANDS THEREFOR

the specification of which:

is attached hereto
☒ was filed in the United States on February 14, 1992 as Application Serial No. 07/835,799 *(for declaration not accompanying application)*
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APPLICATION NUMBER	COUNTRY	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. 119/172
			YES ☐ NO ☐
			YES ☐ NO ☐
			YES ☐ NO ☐
			YES ☐ NO ☐

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201	FULL NAME OF INVENTOR	LAST NAME ARUFFO	FIRST NAME ALEJANDRO	MIDDLE NAME A.	
	RESIDENCE & CITIZENSHIP	CITY Edmonds	STATE OR FOREIGN COUNTRY Washington	COUNTRY OF CITIZENSHIP MEXICO/ITALY	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS 1012 Spruce Street	CITY Edmonds	STATE OR COUNTRY Washington	ZIP CODE 98020
202	FULL NAME OF INVENTOR	LAST NAME LEDBETTER	FIRST NAME JEFFREY	MIDDLE NAME A.	
	RESIDENCE & CITIZENSHIP	CITY Seattle	STATE OR FOREIGN COUNTRY Washington	COUNTRY OF CITIZENSHIP U.S.A.	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS 306 N.W. 113th Place	CITY Seattle	STATE OR COUNTRY Washington	ZIP CODE 98177
203	FULL NAME OF INVENTOR	LAST NAME STAMENKOVIC	FIRST NAME IVAN	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY Brookline	STATE OR FOREIGN COUNTRY Massachusetts	COUNTRY OF CITIZENSHIP SWITZERLAND	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS 59 Babcock Street	CITY Brookline	STATE OR COUNTRY Massachusetts	ZIP CODE 02146
204	FULL NAME OF INVENTOR	LAST NAME NOELLE	FIRST NAME RANDOLPH	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY Plainfield	STATE OR FOREIGN COUNTRY New Hampshire	COUNTRY OF CITIZENSHIP U.S.A.	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS Freeman Hill Road	CITY Plainfield	STATE OR COUNTRY New Hampshire	ZIP CODE 03781
205	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY	ZIP CODE
206	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY	ZIP CODE

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SIGNATURE OF INVENTOR 201	SIGNATURE OF INVENTOR 202	SIGNATURE OF INVENTOR 203
Alejandro A. Aruffo	Jeffrey A. Ledbetter	Ivan Stamenkovic
DATE 1992	DATE 1992	DATE 16th April 1992
SIGNATURE OF INVENTOR 204	SIGNATURE OF INVENTOR 205	SIGNATURE OF INVENTOR 206
Randolph Noelle		
DATE 1992	DATE	DATE 16th April 1992

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

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☒ is attached hereto
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APPLICATION NUMBER	COUNTRY	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. 119/172
			YES ☐ NO ☐
			YES ☐ NO ☐
			YES ☐ NO ☐
			YES ☐ NO ☐

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201	FULL NAME OF INVENTOR	LAST NAME: ARUFFO	FIRST NAME: ALEJANDRO	MIDDLE NAME: A.	
	RESIDENCE & CITIZENSHIP	CITY: Edmonds	STATE OR FOREIGN COUNTRY: Washington	COUNTRY OF CITIZENSHIP: MEXICO/ITALY	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: 1012 Spruce Street	CITY: Edmonds	STATE OR COUNTRY: Washington	ZIP CODE: 98020
202	FULL NAME OF INVENTOR	LAST NAME: LEDBETTER	FIRST NAME: JEFFREY	MIDDLE NAME: A.	
	RESIDENCE & CITIZENSHIP	CITY: Seattle	STATE OR FOREIGN COUNTRY: Washington	COUNTRY OF CITIZENSHIP: U.S.A.	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: 306 N.W. 113th Place	CITY: Seattle	STATE OR COUNTRY: Washington	ZIP CODE: 98177
203	FULL NAME OF INVENTOR	LAST NAME: STAMENKOVIC	FIRST NAME: IVAN	MIDDLE NAME:	
	RESIDENCE & CITIZENSHIP	CITY: Brookline	STATE OR FOREIGN COUNTRY: Massachusetts	COUNTRY OF CITIZENSHIP: SWITZERLAND	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: 59 Babcock Street	CITY: Brookline	STATE OR COUNTRY: Massachusetts	ZIP CODE: 02146
204	FULL NAME OF INVENTOR	LAST NAME: NOELLE	FIRST NAME: RANDOLPH	MIDDLE NAME:	
	RESIDENCE & CITIZENSHIP	CITY: Plainfield	STATE OR FOREIGN COUNTRY: New Hampshire	COUNTRY OF CITIZENSHIP: U.S.A.	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: Freeman Hill Road	CITY: Plainfield	STATE OR COUNTRY: New Hampshire	ZIP CODE:
205	FULL NAME OF INVENTOR	LAST NAME:	FIRST NAME:	MIDDLE NAME:	
	RESIDENCE & CITIZENSHIP	CITY:	STATE OR FOREIGN COUNTRY:	COUNTRY OF CITIZENSHIP:	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS:	CITY:	STATE OR COUNTRY:	ZIP CODE:
206	FULL NAME OF INVENTOR	LAST NAME:	FIRST NAME:	MIDDLE NAME:	
	RESIDENCE & CITIZENSHIP	CITY:	STATE OR FOREIGN COUNTRY:	COUNTRY OF CITIZENSHIP:	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS:	CITY:	STATE OR COUNTRY:	ZIP CODE:

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SIGNATURE OF INVENTOR 201: Alejandro A. Aruffo	SIGNATURE OF INVENTOR 202: Jeffrey A. Ledbetter	SIGNATURE OF INVENTOR 203: Ivan Stamenkovic
DATE: 1992	DATE: 1992	DATE: 1992
SIGNATURE OF INVENTOR 204: Randolph Noelle	SIGNATURE OF INVENTOR 205:	SIGNATURE OF INVENTOR 206:
DATE: 1991	DATE:	DATE: